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REVERSAL OF A SECONDARY SEX CHARACTER BY SELECTION

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I. INTRODUCTION

PAST selection work on quantitative characters, notably using *Drosophila*, has usually been made with equal rigour on both sexes. In the present account numerous lines, from the same F_2, have been differentially selected with respect to the sexes within the same line. Since the effective response to selection depends upon the correspondence between genotype and phenotype, any sexual difference in the correlation between genotype and phenotype would result in selection acting with different speeds in the two sexes.

The failure of the phenotype to mirror exactly the genotype in all individuals from a culture means that in many selections individuals are selected which are genetically lower or higher than expected. If a mid level is required, this inaccuracy becomes more apparent than if high or low selections are being considered. In the case of extreme selections the direction is definite and any inaccurate selections will not affect the next generation so markedly, for the top or bottom stratum of the population is already represented in the selected individuals. In a mid selection there may be slight inaccuracies in selection. For example the environment may affect one or other level preponderantly and thus offer for selection individuals which are mostly from one extreme of the population and will result in a gradual drift of the mean. This implies directional interaction of genotype and environment. In general, mid-lines may be expected to drift more than the extremes but to drift erratically without specific and continued direction. In the selections under review the best methods for mid level continuation are demonstrated.

The fertility of selected lines is usually impaired by continued successful selection. Selection for particular genotypes will undoubtedly disturb the normal good fertility to some extent by correlated response but as most selections also tend to inbreed the population these two factors must act concurrently. The difference in rigour among the twelve selections gives an opportunity to study the effects on fertility of selection, for inbreeding was constant in all twelve lines.

2. EXPERIMENTAL METHOD

Selection was practised on the abdominal chaetæ of *Drosophila melanogaster* described by Mather, 1941. Two inbred lines, Oregon + (inbred for 250 generations) and Samarkand + (inbred for 150 generations) were crossed reciprocally and a large F_2 was made up from the two reciprocal F_1's. The variously selected lines were taken from the F_2. The selections, apart from the F_2 when 80 flies of each sex were counted, were made on counts of 20 ♀♀ and 20 ♂♂. Two cultures, one a reserve, were made from each line using 2 ♀♀ and 2 ♂♂. The reserve was never used unless the principal culture failed, which was seldom. Variability, therefore, was not preserved to the same extent as in the method employed by Mather and Harrison (1949) and the response cannot, in consequence, be expected to be so great.

The whole experiment was repeated later using the same inbred material and using the same technique in order to assess more accurately the effects of the different selective methods. So much seems to depend on the accuracy of genotype selection in the first few generations that for a proper assessment of the responses several replicates would have been preferred but, due to the prodigious labour this would have involved, only two were done.

The twelve selected lines are listed below together with their symbols which will be used throughout this account :—

♀♀ selected	♂♂ selected	Symbol
High	High	HH
High	Mid *	HM
High	Low	HL
Mid	High	MH
Mid	Mid	MM
Mid	Low	ML
Low	High	LH
Low	Mid	LM
Low	Low	LL
Mid	Alternating High and Low †	M (H and L)
Alternating High and Low	Mid	(H and L) M
Alternating High and Low	Alternating High and Low	(H and L) (H and L)

* The mid selection is at the mean of the particular sex in the culture counted at that generation.

† Selection alternates each generation, *i.e.* first generation of selection was high, second low, third high, etc. ♂♂ and ♀♀ both alternated in the same direction at the same time.

3. CULTURE VARIATION

It has already been pointed out that inaccuracies in selection are likely to occur in a highly variable culture. This is due to developmental differences within a culture which affect the expression of chaeta number to some extent. A comparison of the variances of a group of F_2 means with the mean of the same F_2 variances shows, however, that the difference between cultures is relatively low, while the variation within a culture is, as expected, much higher. This shows that the variation due to unknown environmental causes is

due in the main to variation between individuals in a culture and not to variation between the cultures. A little variation between cultures does occur, as demonstrated by Mather, 1949.

TABLE 1

Mean chaeta number of 11 F_2's (Or. × Skd) (20 ♂♂ counted in each class)		Mean squares on corresponding groups of the 20 ♂♂	
1. 34·55	Mean square *0·268*	4·137	Average mean square *5·945*
2. 34·75		6·421	
3. 34·95		2·158	
4. 34·85		5·421	
5. 34·5		4·158	
6. 35·1		4·158	
7. 33·35		8·947	
8. 33·55		12·263	
9. 33·7		6·316	
10. 34·7		4·211	
11. 34·55		7·211	

Temperature and culture condition do not affect the expression of chaeta number very greatly but the unknown developmental differences can give considerable differences in the variances. Culture 3 has a low variance of 2·158, but culture 8 gives an estimate of 12·263. The selective sieve will doubtless vary in its efficiency if the individuals within the cultures can vary so greatly. It is the development of the individuals within a culture that is, in some cases, masking genetic differences between individuals. Between lines, which is the comparison in which we are interested, the quirks of development may be expected to cancel each other out and the comparison of culture means is therefore a reliable appraisal of the genetic conditions prevailing in the line.

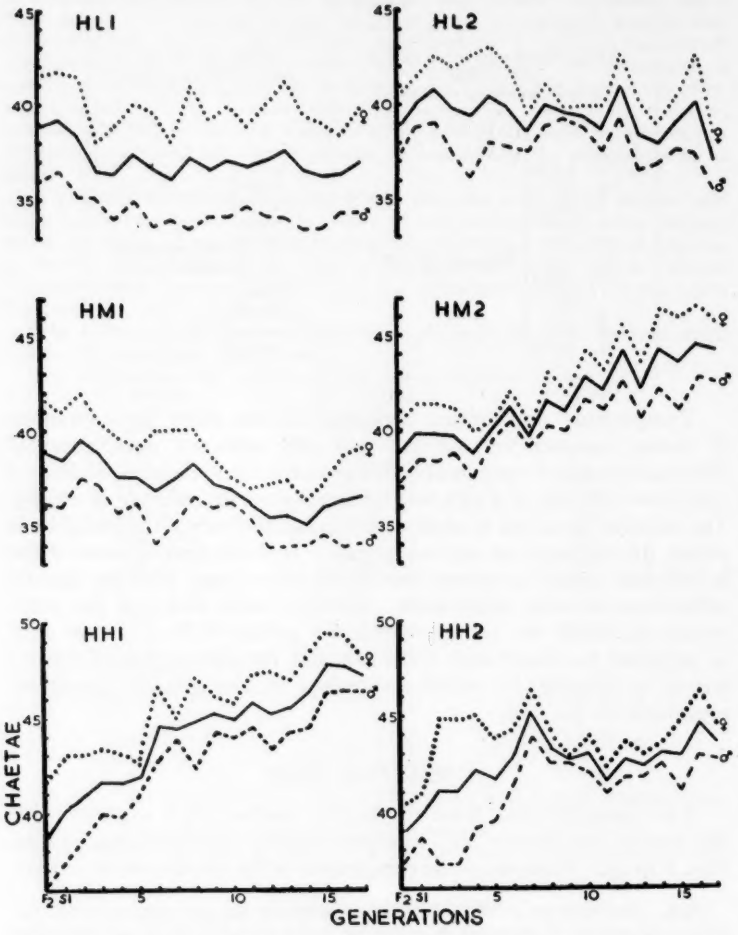
4. SELECTION LINES

The complete data from the twelve selected lines together with the repeat experiment are presented in the accompanying graphs (figs. 1 to 4). These show the comparison of the duplicated selections.

HH. The response in HH1 (the first experiment) was the same until S7 (the seventh generation of selection) as in the line in the second experiment, hereinafter called HH2. But HH1 rose slowly after S7 while HH2 failed to respond at all after S7.

HM. These two lines responded very differently. HM1 fell slightly until S12 and then stabilised itself, while HM2 rose steadily for the whole 17 generations. This comparison is an object lesson in the way in which two similarly selected lines follow different paths. This difference in response may be due either to the different genetic potentialities of the first parents chosen or to the difference in the genetic make-up of the mid males, which being most heterozygous, are perhaps most likely to vary in response according to the vagaries of recombination while

some might fail to respond owing to chance homozygosity for recombinations of mid effect. One other possibility may be mentioned, namely that chromosomal selection of one or other set of inbred chromosomes might have been selected before recombination took place in HM1 thus minimising the polygenic variability available



FIGS. 1–4.—The response of the various selections. Solid lines show the means of the sexes; dotted lines the females and broken lines the males.

for release. The reciprocal selection, MH, also responded differently in the two experiments while all other lines were on the whole similar.

HL. HL1 fell a little until S3 and stabilised itself for the remaining 14 generations. HL2 on the other hand rose a little and then fell slightly. In these two opposed selections the pull between selected ♂♂ and ♀♀ was about equal for apart from the first and perhaps the last few generations the lines did not respond.

MH. It was mentioned in the reciprocal selection, HM, that the divergence of the two experiments is perhaps greatest in this type of selection. Whether this divergence is consequent upon the type of selection or merely fortuitous is unproved. MH1 rose steadily until S9 and from there remained fairly stable. MH2, however, remained stable throughout the seventeen generations of selection.

MM. MM1 died at S_4 and had to be remade from M (H and L) ♀♀ and

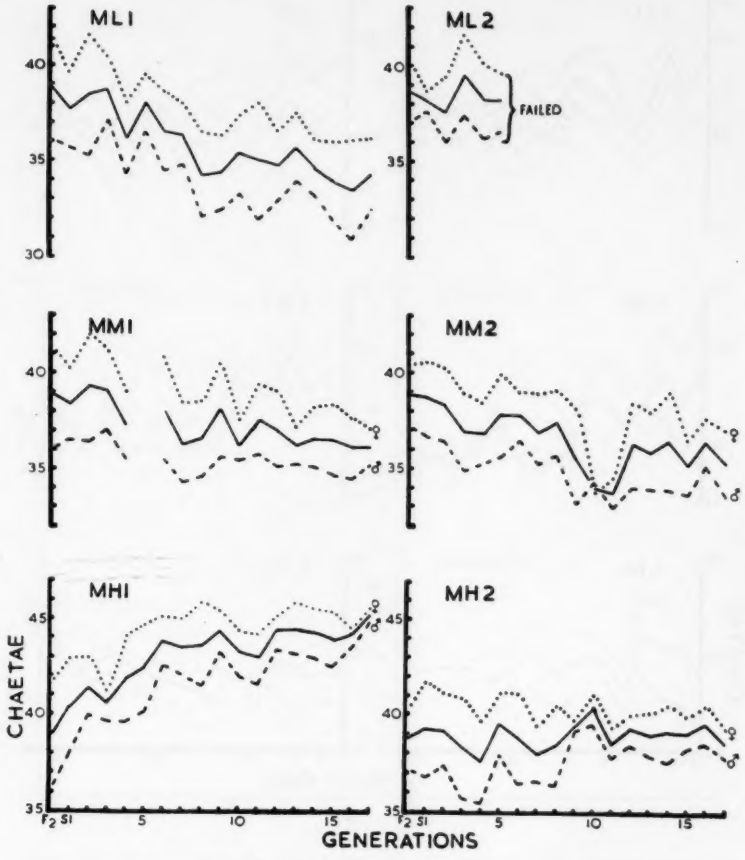


FIG. 2.

(H and L) M ♂♂ obtained from S6 cultures. These two lines were thought to approximate as near as possible to the MM selection. The mean of MM2 and the repeat of MM1 drift down steadily though slowly for the run of the experiment. It would seem that for a stable mid line the MM type of selection is not the most satisfactory.

ML. ML1 responded in a low direction throughout the selection. Here the power of the low selection on the ♂♂ as compared with the mid selection on the ♀♀ cannot be effectively established as ML2 died prematurely at S6. However,

it appears from the other lines in which a mid selection was used that whenever the other sex was selected in any way other than in a high direction the line never responded in a + direction. The mid selection, therefore, would seem incapable of giving a high boost to the selection.

LH. In this opposed selection there is closer agreement between the replicates than in the reciprocal selection, HL. There is very little departure from the point

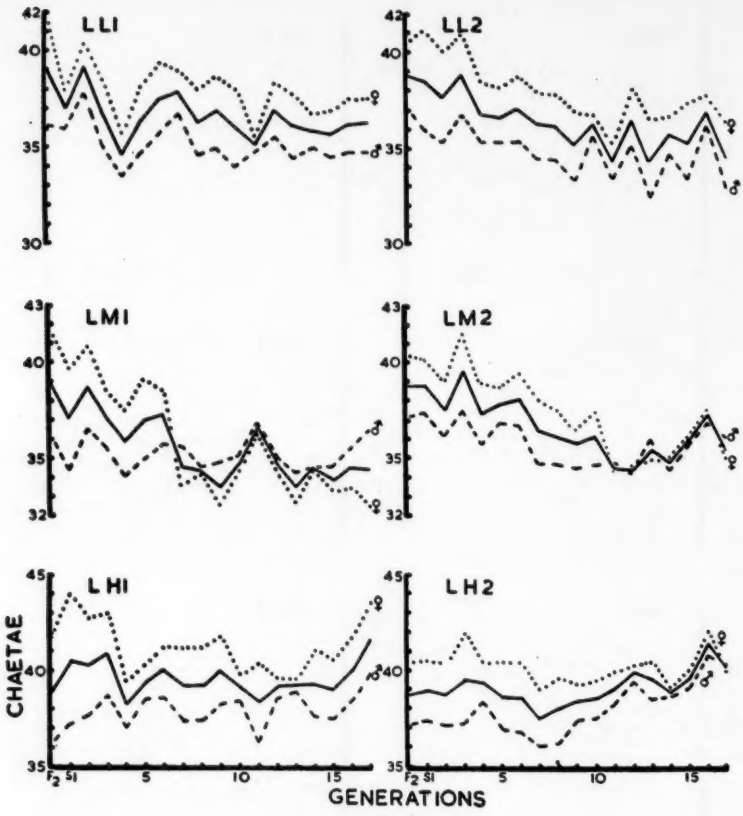


FIG. 3.

of commencement and it seems that the low female selection and the high male selection have balanced each other. No greater release or build-up of high or low polygenic complexes has therefore been favoured in this set.

LM. The LM lines both responded in a low direction and were equal in response to the LL selected lines. LM1 and LM2 both showed a curious reversal in the number of chaetæ produced by ♂♂ and ♀♀. This phenomenon will be dealt with in greater detail later in the account.

LL. Both lines responded similarly, LL2 responding a little more than LL1. No increased response in a low direction occurred after S11 when both lines become stable. The low level reached by the LL lines was no more than that reached by many other selections under discussion.

M (H and L). These two lines responded in a similar fashion to the LL lines except that no more response occurred after S7. Here it is evident that in this material a low response is easier to obtain than a high.

(H and L) M. The first line of this selection remained at the mid level throughout the seventeen generations of selection with some fluctuations. (H and L) M2 remained at a slightly lower level, except for a rise at S6 and S17.

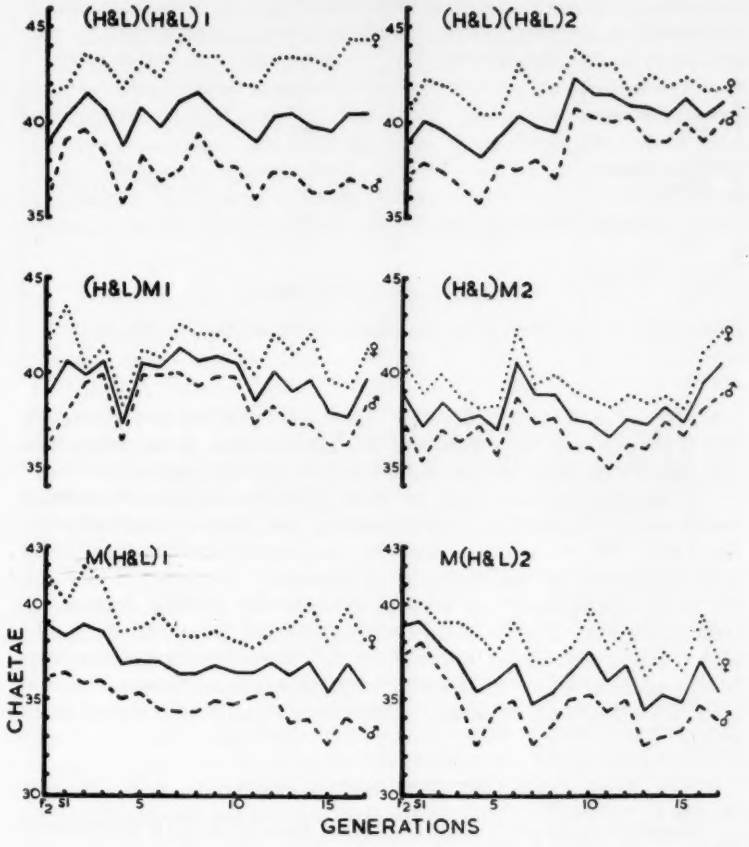


FIG. 4.

(H and L) (H and L). The first line remained about the mid level throughout while (H and L) (H and L)2 was a little higher towards the end of experiment following a rise at S9.

If we summarise the results of these various selections further we have those which responded and those which remained unchanged in both experiments, in the first experiment only and in the second experiment only.

Although it is possible to hazard some conclusions from table 2 it must be emphasised that they rest on two sets of data only. The overall responses are small and this is unfortunate for it does not allow an accurate assessment of line differences. If we consider the selections in the first experiment then we could say that the ♂♂ are

TABLE 2

	In expt. 1 and 2	In expt. 1 only	In expt. 2 only
Good response High	...	MH HH	HM
Slight response High	...	...	HH (H and L) (H and L)
Good response Low	LM, M (H and L) LL	ML	...
Slight response Low	MM	HM, HL	...
Mid level	LM, (H and L) M, LH	(H and L) (H and L)	HL, MH

less efficient in the production of a high chaeta number under selection but if we pool the data from the two experiments, as we must, then this sexual difference in the response to selection disappears.

If the sexes do not differ in their ability to respond to selection which seems to be the case from these data, then in an opposed selection like LH or HL the relative ability to release plus or minus variability must be shown by the direction of response. Neither LH nor HL (in both experiments) can be said to show any striking response in either direction. The double alternating selection (H and L) (H and L) also remains near or at the mid level. These results indicate that in this material no more release of variability is found in one direction over the other and show that selection is equally effective in both sexes.

5. FERTILITY OF THE LINES

Fertility counts were made on all the lines in the first experiment. This was measured by taking the total number of flies emerging from the culture. The figures listed below are averages of flies per bottle of the lines over the whole seventeen generations.

LL	280	ML	230
HH	255	(H and L) M	210
M (H and L)	230	MH	190
LH	230	HM	180
LM	230	HL	175
(H and L) (H and L)	230	(MM not comparable due to line being remade so omitted)	

The fertility of all the lines was good in both experiments and from the fertility figures obtained from expt. 1 it appears that the extremes of selection (*i.e.* HH and LL) are not deleteriously affected by selection any more than any of the other lines, in fact they head the list for good fertility. Any selection, therefore, is just as likely to upset the fertility balance as an extreme type of selection. The inbreeding of a selected stock may therefore have a considerable effect on the fertility apart from the fertility unbalance due to selection.

A comparison of the present chaeta responses with those of Mather and Harrison (1949) shows the present ones to be much lower even though the original cross was the same. As the responses were small it may be that there was not such a great correlated effect on fertility. This difference in response between the two experiments may indicate a change in the genetic make-up of Samarkand and/or Oregon inbred since its use by Mather and Harrison some eleven years earlier.

6. SEX DIFFERENCES IN CHAETA NUMBER

Normally the females have more abdominal chaetæ on the ventral surfaces of 4th and 5th segments than the males. The sex difference in chaeta number varies according to the total number of chaeta. Fig. 5 illustrates this effect. The data used for fig. 5 are obtained from the many levels obtained in the selections of Mather and Harrison (1949), which originated from the same material that was again used in this experiment, namely Samarkand × Oregon. The levels of Mather and Harrison were all established by a LL or an HH type of selection.

The relationship of sex difference to total chaeta number gives us a yard stick by which we can measure any aberrant difference. In fig. 6 we insert the regression line and limits obtained in fig. 5 and superimpose the sex differences occurring in the selection lines under discussion. Many points lie outside the limits of fig. 5 which may be due to the small effects operating on the sexes consequent upon the various selection methods. The path of regression employed is too steep or too high in overall position for the present data, for the HH lines of the present experiment fall below lowest values in fig. 5 though the sex mean is well within range. However, it appears that the spread of the variously selected lines is much greater than expected from previous experience and that the LM lines are extremely aberrant.

Sex difference has already been shown to vary with slight genetic alterations (Harrison and Mather, 1950), but never to the extent of that shown by the LM lines (fig. 3). The LM selections show a complete reversal, the females carrying fewer chaetæ than the males. This reversal, however, is due not to equal changes in both sexes but only to the females which carry fewer than normally. The males remained constant throughout both experiments. In LM1 when the

females became lower than the males at S7 the emergence of the females became about one half that of the males. In LM2 the emergence of the males and females was equal. Blackened larvæ and pupæ present in LM1 cultures were presumably the females which had failed to emerge.

If in the LM1 line crossing over had occurred between the X and Y chromosomes (Mather, 1944 ; Philip, 1935) then the females homozygous for the recombinant chromosomes may have been lethal while the heterozygous recombinant females showed the reduction

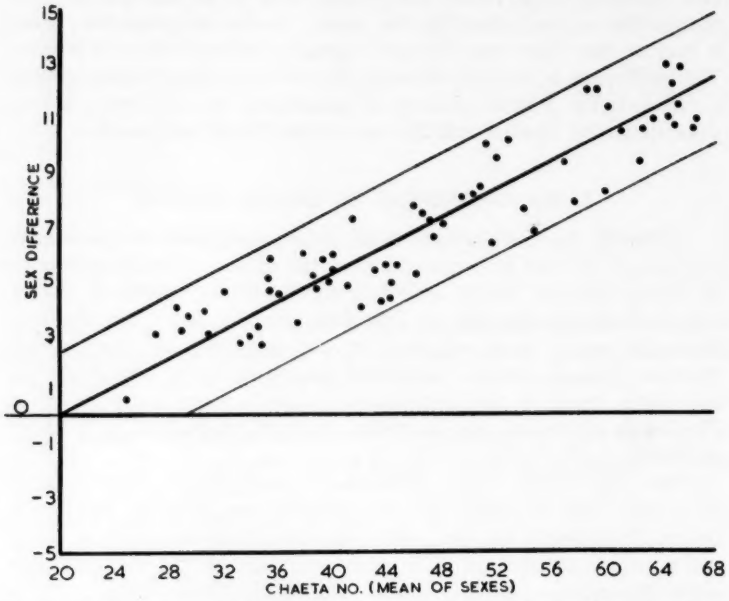


FIG. 5.—The relation between high and low chaeta number and the sex difference. (Data obtained from "Manifold Effect and Selection," K. Mather and B. J. Harrison, 1949.)

in chaeta number. The Y chromosome has been shown to be slightly active but due to its comparative inertness the recombinant would be expected to be less active polygenically than the normal X. The low female selection in these LM lines would favour the new recombinant X chromosomes while the hemizygous males with a recombinant X were not so markedly affected, the mid selection on the males not being strong enough to pick out recombinant Y chromosomes. That the phenomenon occurred again in the LM2 line may be said to be possibly fortuitous for the type of selection favouring recombinant females existed in the LH lines but no sex differences of the LM type occurred in LH1 but in LH2, towards the end of the experiment the sex reversal began to appear. In LH2,

however, the reversal was due to the males increasing in chaeta number while the females remained at the same level. It appears to be a possibility then that the LH2 selection was favouring recombinant Y chromosomes while the selection had failed to pick out low chaeta producing recombinant X chromosomes. Had this occurred the sex reversal would doubtless have been considerable.

One other explanation for the alternation of the sex differences remains to be mentioned. It is possible that due to the sexually opposed selections practised here genes have been selected which

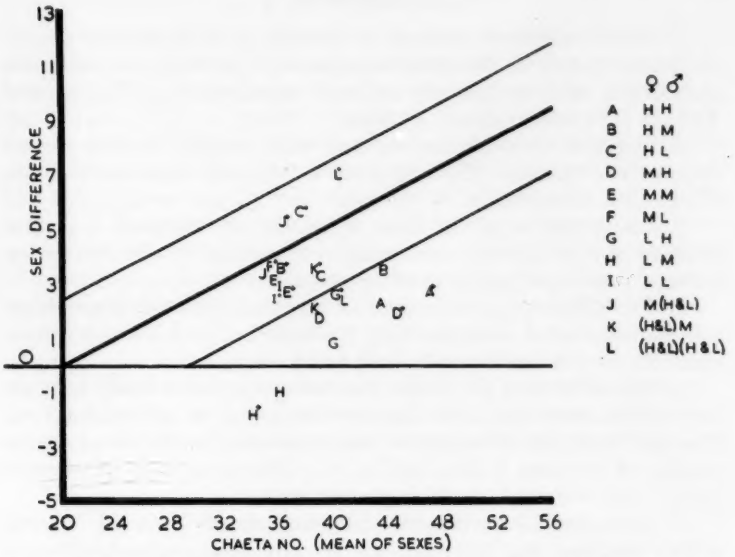


FIG. 6.—The sex differences obtained from the present selection lines plotted on the path of regression obtained in Fig. 5. Letters and plus sign indicate results from Experiment 1 while plain key letters indicate Experiment 2. The positions are obtained by pooling results of the last five generations of selection (*i.e.* S 13–S 17).

manifest themselves differently in the two sexes. The fact that they did not show themselves in all selections favouring such a situation must similarly be attributed to inaccuracies of selection. The poor emergence of females in LM1 may be due in this case to a correlation of chaeta genes, sex limited in expression, and genes for poor viability. Where the correlation did not exist, or was not so complete, females emerged and exhibited the sex reversal in chaeta number.

The sex differences vary materially between many of the lines. In (H and L) (H and L)1 the difference is large throughout the experiment, while in (H and L) (H and L)2 the difference diminishes after S8. HL1 also shows a large difference while the rest of the lines, apart from the LMs are either less constant or the difference

is small. It is apparent that sex differences can be altered by selection but the mechanism by which they are obtained can only be conjectured here.

The similarity of response of means in reciprocal selections shows that selection is equally effective in both sexes. The changes in sex differences, however, show that it is possible to get sex-limited responses to selection. This is presumably a way sexual selection in nature produces changes in secondary sex characters.

7. SUMMARY

1. Twelve different methods of selection have been tried on the abdominal chaetæ in *Drosophila melanogaster*. The sexes were subjected to opposed, and to partially opposed, selections, *e.g.* ♀♀ low and ♂♂ high ; ♀♀ mid level and ♂♂ high.

2. Opposed selections on the two sexes resulted in little or no response to selection. The best method for a mid level continuation of a stock is considered.

3. The fertilities of the lines tested did not indicate a greater decrease in fertility with a more rigorous method of selection owing perhaps to the total smallness of the responses.

4. The difference in behaviour of the duplicated selections shows the caution needed in interpreting the results of an isolated selection when comparisons of treatments are being made.

5. Sex differences in chaeta number vary considerably between lines and in some cases seem to be correlated with the type of selection. The relation of sex differences in chaeta number and the mean chaeta number of the sexes is disturbed by the different methods of selection when compared with an arbitrary standard.

6. Normally, ♀♀ have more abdominal chaetæ than the ♂♂, but in two selections the difference has actually been reversed. Thus a sex reversal in a secondary sex character has been obtained by selection. These reversals can equally well be explained by assuming crossing-over between the X and Y chromosomes, or selection for sex-limited genes.

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THE PROGRESS OF INBREEDING WHEN HOMOZYGOTES ARE AT A DISADVANTAGE

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I. INTRODUCTION

WHEN an individual, homozygous for one or other of two alleles of a gene for which the population is heterogenic, mates with another bearing to it a relationship closer than the average of the population, the offspring will in general include a greater proportion of such homozygotes than would the offspring of a mating of average relationship. Heterozygotes, on the other hand, give half heterozygotes and half homozygotes among their offspring, no matter what their mates may be. Thus inbreeding leads to a fall in the proportion of heterozygous offspring of homozygotes, without any change in the proportion of homozygous offspring of heterozygotes. The proportion of homozygotes will therefore increase in the population and, as it increases, we should expect the process to continue and the chance of any offspring of a homozygote being itself homozygous also to increase. So, mutation apart, the outcome of sufficiently close inbreeding will in the long run be complete homozygosis. The same result will, of course, follow where more than two alleles are present in the population.

The approach to homozygosis depends on the number of homozygotes produced by heterozygous parents exceeding any loss of homozygotes brought about by crossing or indeed by any other means. Of other means, mutation will in general be unimportant. It may result in the final state falling slightly short of complete homozygosis ; but the proportion of heterozygosis maintained by mutation pressure must be small unless the gene has an unusually high mutation rate. It is worth noting that any mutation will tend to cause heterozygosis in inbred populations. This will be as true where both alleles are equally mutable as where the mutation rate of one exceeds that of the other. The effect of a difference in the mutation rates will be seen in a drift towards the preponderance of one allele in the population, a preponderance depending finally on the relative mutation rates of the alleles and not on their initial frequencies. With equality of mutation rates, on the other hand, the initial gene frequencies will be maintained apart from random variations. The change under unequal mutation rates must in any case be very slow, detectable only over very large numbers of generations and quite negligible in ordinary inbreeding experiments.

Mutation by itself may therefore be neglected in considering the outcome of inbreeding, except perhaps in special cases. The effects of selection, on the other hand, can be far from negligible. Where one allele is at an advantage, but heterozygotes as such are not, homozygosis will ensue at a speed depending on the system of inbreeding. The homozygotes for the favoured allele will then finally oust those for the less favoured at a speed depending on the selection pressure, and usually below that of the progress of inbreeding. With the heterozygotes at an advantage over both homozygotes, however, the whole course and even the outcome of the inbreeding will be altered, and altered profoundly if the selective differential is high enough. For, if the loss of homozygotes by selection is not less than their accretion from heterozygous parents, the proportion of homozygotes in the population will not increase in the population. The progress of the inbreeding will be prevented and heterozygotes will be maintained in the population. Inbreeding and selection will come to balance and annul one another's effect.

2. GENERAL CONSIDERATION

In calculating the effect of selection favouring heterozygotes on the constitution of inbred populations we shall confine our attention to single genes. The results will, of course, apply to members of sets of genes affecting the same character provided they do not interact in producing their effects.

The problem may be divided into four stages.

(i) The complete description of the population when a particular mating system is in operation.

(ii) The investigation of the way this description changes from one generation to the next in the absence of selection.

(iii) The introduction of a parameter $x (0 \leqslant x \leqslant 1)$ as the average survival rate of the homozygotes compared with the heterozygotes. Initially x is assumed to be the same for all homozygotes.

(iv) The description of the equilibrium population attained for various values of x.

Six mating systems will be considered, *viz.* selfing, sib mating, parent-offspring mating, half-sib mating, double first-cousin mating and mating at random. Random mating, though not an inbreeding system, is included for the purpose of comparison with the others. A system of mixed selfing and random mating behaves in a similar way under selection against the homozygotes, but, as its mathematical treatment is different, it will be considered in a separate paper.

It will be convenient to deal first with some general points and results.

Consider the genotypes of individuals in a population containing

a certain gene with any number of alleles. As the mating system becomes more complex, so do the groups of individuals (mating units) by which the population is naturally classified. Under selfing we are concerned with the proportions of homozygotes and heterozygotes ; under sib mating with the various mating couples ; under double first-cousin mating with pairs of mating couples. In a large population and for the first five of the above systems, the frequencies of these groups in a generation are linearly related to those of the previous generation, so that the changes in frequency from generation to generation may be completely specified by a matrix, called the generation matrix of the system (Fisher, 1949).

The parameter x is introduced by multiplying the rows of the generation matrix by suitable powers of x, and dividing the columns by suitable compensating linear forms in x. The maximum latent root of the resulting matrix determines the final stable equilbrium population, but, as these latent roots are functions of x, different ones, determining differing types of equilibrium, may be the greatest for different values of x.

In the absence of selection ($x = 1$), the final result of continuing with any of the five inbreeding systems is homozygosis ; the population splits into sub-populations of close relatives (families) each containing only one allele of the gene under consideration. Below some lower value of x the final stable state is a population containing some heterozygotes, each family containing two alleles of the gene. With more complex mating systems populations whose families contain three alleles may be stable at some still lower value of x. However, in any range of x in which there are n alleles in families in the stable state, families containing $m<n$ alleles can exist, but only in unstable equilibrium.

Stable equilibrium and unstable equilibrium have the following meanings. In the former, if extra alleles are introduced (through a mating irregularity or mutation) the family will in time split into sub-families containing the original number of alleles. In the latter, extra alleles up to n-m in number, once introduced, will tend to remain in the family which will in time adjust its frequencies to a new (stable or unstable) equilibrium. Both of these types of equilibrium are stable to the arbitrary introduction into a family of extra individuals carrying alleles already in the family—in time the same equilibrium will be re-established.

Linkage has no effect on the equilibrium state of a gene, but it may seriously upset the rate of approach to equilibrium. Linkage effects are ignored in this paper.

3. THE MATING SYSTEMS

3.1. Selfing.—For any number of alleles $a, b, c, \ldots$ of one gene, the frequencies p and q ($p+q=1$) of all homozygotes and of all heterozygotes

become $(p+\frac{1}{2}q, \frac{1}{2}q)$ in one generation. The generation matrix is thus

		Parents	
		aa	*ab*
Offspring	*aa*	1	$\frac{1}{2}$
	ab	·	$\frac{1}{2}$

where *aa* denotes all the homozygotes *aa*, *bb*, ... and *ab* the heterozygotes *ab*, *ac*, *bc*, ... It is assumed that the population is sufficiently large for random variations to be negligible. If all the heterozygotes have the same viability and if only x of any of the homozygotes survive for each one of the heterozygotes, the effects of reduction in viability operating between and within families with the same effect (*e.g.* competition between members of different progenies being as strong as between members of the same family), the matrix becomes

$$\begin{bmatrix} x & \frac{1}{2}x \\ \cdot & \frac{1}{2} \end{bmatrix}$$

The latent roots are x and $\frac{1}{2}$ and these determine equilibrium populations (1, 0) and $(x, 1\text{-}2x)$ * respectively. After many generations the population is that determined by the maximum latent root.

For $x \geqslant \frac{1}{2}$ homozygosity results.

For $x < \frac{1}{2}$ some heterozygosity always remains (if it was present in the original population. Otherwise an unstable homozygous state is possible).

After n generations the frequencies (p_n, q_n) of homozygotes and heterozygotes are given by

$$p_n \propto x^n\left(p - \frac{qx}{1-2x}\right) + 2^{-n}\frac{px}{1-2x}$$

$$q_n \propto 2^{-n}\, q$$

If we start with a completely heterozygous population $(p, q) = (0, 1)$, the frequency of heterozygotes remaining after n generations of selfing

$$= h = \frac{1-2x}{1-x-2^n x^{n+1}} \qquad \text{for } x \neq \tfrac{1}{2}$$

$$\text{or} = \frac{2}{2+n} \qquad \text{for } x = \tfrac{1}{2}$$

* While (p, q) is taken to be such that $p+q = 1$, it is convenient at times to use (u, v) such that u and v are only proportional to the population frequencies. This need cause no trouble since where necessary we can divide through by $(u+v)$.

so that as n becomes very large

$$h \to 0 \qquad \text{for } x \geqslant \tfrac{1}{2}$$

$$\text{or} \to \frac{1-2x}{1-x} \qquad \text{for } x \leqslant \tfrac{1}{2}.$$

Fig. 1 depicts the approach to equilibrium starting from complete heterozygosity ($n = 0$) or from a random mating population containing two alleles ($n = 1$). Near $x = \frac{1}{2}$ the rate of approach to equilibrium is very slow.

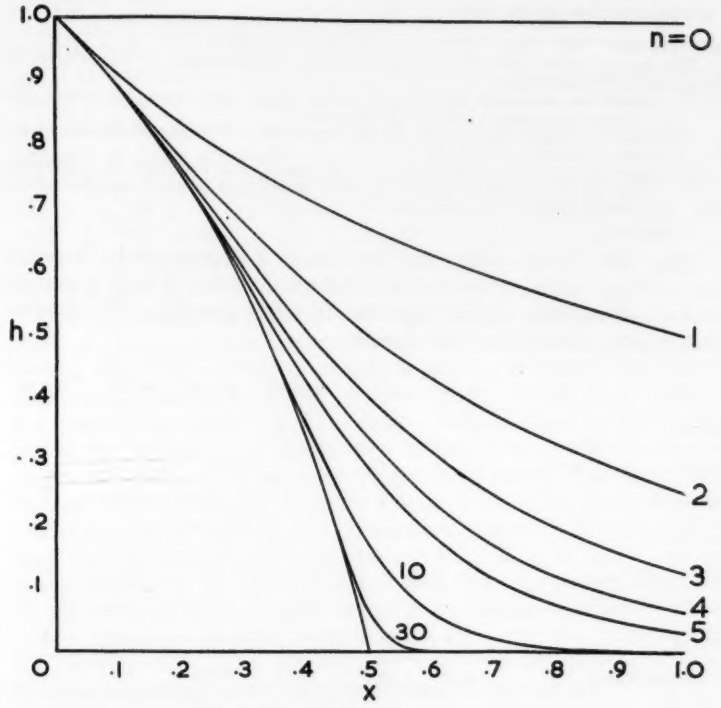


FIG. 1.—Approach to equilibrium under selfing.

h = proportion of heterozygotes in the population.

x = relative survival rate of the homozygotes, the survival rate of the heterozygotes being taken as 1.

n = number of generations of selfing starting from an entirely heterozygous population ($n = 0$) or from a random mating population containing only two alleles ($n = 1$).

If we put $d = 100(1-x)$, d is the percentage disadvantage of the homozygotes compared with the heterozygotes. Column (i) in table 1 contains, for each of the five systems, the value of d above which heterozygosity is possible. These figures are larger than those

given erroneously in a previous paper (Mather and Hayman, 1952) on the same topic.

TABLE 1

	i	ii	iii
Selfing (S)	50·0	66·7	50·0
Sib mating (Sb)	23·7	44·6	34·0
Parent-offspring mating (PO1)	19·8	39·9	15·2
" (PO2)	25·0	47·2	34·6
Half-sib mating (HSb)	18·8	...	30·9
Double first-cousin mating (DFC)	14·5	...	23·2

The figures in this table are percentage selective disadvantages of homozygotes as compared with heterozygotes.

(i) Shows the maximum disadvantage under which the inbreeding system will eventually produce complete homozygosity.

(ii) Shows the disadvantage at which the population will eventually become 50 per cent. homozygous (see *4.3*).

(iii) Shows the maximum disadvantage of one homozygote for which the population will become homogenic when the other homozygote is lethal, only two alleles being present initially (see *4.3*).

3.2. Sib mating.—Here the population is appropriately classified into mating couples. Up to four alleles may occur in such a mating unit of which seven distinct types are therefore possible. The generation matrix, allowing for selection, is

	$aa\times aa$	$aa\times ab$	$ab\times ab$	$aa\times bb$	$ab\times ac$	$aa\times bc$	$ab\times cd$
$aa\times aa$	x	$\frac{1}{4}x^2$	$\frac{1}{4}x^2$	.	$\frac{1}{4}x^2$	.	.
$aa\times ab$	.	x	x	.	x	.	.
$ab\times ab$	.	$\frac{1}{2}$	$\frac{1}{2}$	1	$\frac{3}{4}$	$\frac{1}{2}$	$\frac{1}{4}$
$aa\times bb$	.	.	$\frac{1}{4}x^2$	.	.	.	.
$ab\times ac$	.	.	.	.	$\frac{1}{2}$	$\frac{1}{2}$	$\frac{1}{2}$
$aa\times bc$	.	.	.	.	$\frac{1}{4}x$	.	.
$ab\times cd$	.	.	.	.	.	.	$\frac{1}{4}$
Column divisors	1	$x+1$	$x+1$	1	$x+3$	1	1

To show how this is derived, take the second column for example. Without selection the progeny of $aa\times ab$ will give rise to the three types of crosses $aa\times aa$, $aa\times ab$, $ab\times ab$ in the frequencies $\frac{1}{4}:\frac{1}{2}:\frac{1}{4}$. If only x of the homozygotes survive, these frequencies become proportional to $\frac{1}{4}x^2:\frac{1}{2}x:\frac{1}{4}$. Now if x of the progeny of $aa\times aa$ survive, $\frac{1}{2}(x+1)$ of the progeny of $aa\times ab$ will survive, so that $\frac{1}{4}x^2$, $\frac{1}{2}x$, $\frac{1}{4}$ must each be divided by a factor, $\frac{1}{2}(x+1)$, to make their total equal to $\frac{1}{2}(x+1)$. When $x=1$ this matrix reduces to that given by Fisher (1949).

This matrix provides a good illustration of some general theory.

A mating unit containing n alleles (an n-unit) can only give rise to units containing $m \leqslant n$ alleles. Hence the generation matrix contains sub-matrices (delimited by dotted lines) along the leading diagonal below which only zeros occur. The characteristic equation of the generation matrix is thus the product of the characteristic equations of these sub-matrices. Let λ_{ni} be the ith latent root of the nth sub-matrix in order down the diagonal (under selfing $\lambda_{11} = x$, $\lambda_{21} = \frac{1}{2}$), λ_n the greatest of the λ_{ni} and V_{ni}, V_n the corresponding latent vectors. Then the elements of V_{ni} corresponding to m-units ($m > n$) are evidently zero.

Now two standard results, (i) that the maximum latent root of a matrix with non-negative elements is real and lies between the greatest and least of the column (or the row) totals (and is actually unity if each column totals unity), and (ii) that the elements of the corresponding latent vector are real and of the same sign, may be applied to any sub-matrix of the generation matrix. Hence the elements of V_n corresponding to n-units have the same sign, while if $\lambda_n > \lambda_m$ (all $m < n$) all the elements of V_n have the same sign. Any such λ_n will therefore define a real population equilibrium. If further $\lambda_n > \lambda_m$ (all $m \neq n$) then the equilibrium is stable; but if $\lambda_n < \lambda_m$ (some $m > n$) the equilibrium is unstable.

It only remains to determine the regions in the range of x in which each of the λ_n becomes the maximum for the whole matrix. Under selfing, sib, parent-offspring, and (it is presumed) half-sib and double first-cousin mating, λ_1 is the greatest near $x = 1$, λ_2 is the greatest for some lower values of x, λ_3 at some still lower values, and so on as far as the complexity of the mating system allows. This is not true in general, because for an inbreeding system weaker than first-cousin mating full homozygosis is never attained, *i.e.* λ_1 is never the only maximum latent root of the generation matrix. However, there may still be a sequential appearance of λ_n, λ_{n+1}, λ_{n+2} . . . as the maximum latent root as x decreases from 1 to 0. In the first three of the systems under consideration only families containing either 1 or 2 alleles are possible, depending on the value of x. For half-sib and double first-cousin mating, we have not determined whether families containing more than two alleles can exist or not.

To return to sib-mating, the characteristic equation is

$$(\lambda - x)\{4(x+1)^2\lambda^3 - 2(x+1)(2x+1)\lambda^2 - x^2(x+1)\lambda + x^3\}$$
$$\{(2\lambda - 1)(2(3+x)\lambda + x)\}(4\lambda - 1) = 0$$

Hence $\lambda_1 = x$ and $V_1 = (1\,;\,0\,;\,0\,;\,0\,;\,0\,;\,0)$ corresponding to homozygosis. Let λ_{21}, λ_{22} and λ_{23} be the roots of the cubic. Then for any λ_{2i} and the corresponding value of x

$$V_{2i} = \left(\frac{\lambda x^2}{\lambda - x}\{(1+x)\lambda + x\}\,;\; 4x(1+x)\lambda\,;\; 4(1+x)\{(1+x)\lambda - x\}\lambda\,;\right.$$
$$\left. x^2\{(1+x)\lambda - x\}\,;\; 0\,;\; 0\,;\; 0\right)$$

As x varies from 0 to 1, each λ_{2i} varies monotonically over the range given in the accompanying table.

x	λ_{21}	λ_{22}	λ_{23}
0	$\frac{1}{2}$	0	0
1	$\frac{1}{4}(1+\sqrt{5})$	$\frac{1}{4}$	$\frac{1}{4}(1-\sqrt{5})$

Evidently $\lambda_{21} = \lambda_2$

$$\lambda_{31} = \tfrac{1}{2}$$

$$\lambda_{32} = -\frac{x}{2(3+x)} \qquad \text{so that } \lambda_{31} = \lambda_3$$

$$\lambda_4 = \tfrac{1}{4}$$

Both λ_3 and λ_4 are $\leqslant \frac{1}{2} \leqslant \lambda_2$ for all x in 0 to 1 so that we are only interested in the relative magnitudes of λ_1 and λ_2. At $x = 1$, $\lambda_1 > \lambda_2$ and at $x = 0$, $\lambda_2 > \lambda_1$. The changeover occurs when $\lambda = x$ in the cubic, which then becomes $4x^3+3x^2-2x-2 = 0$ of which the

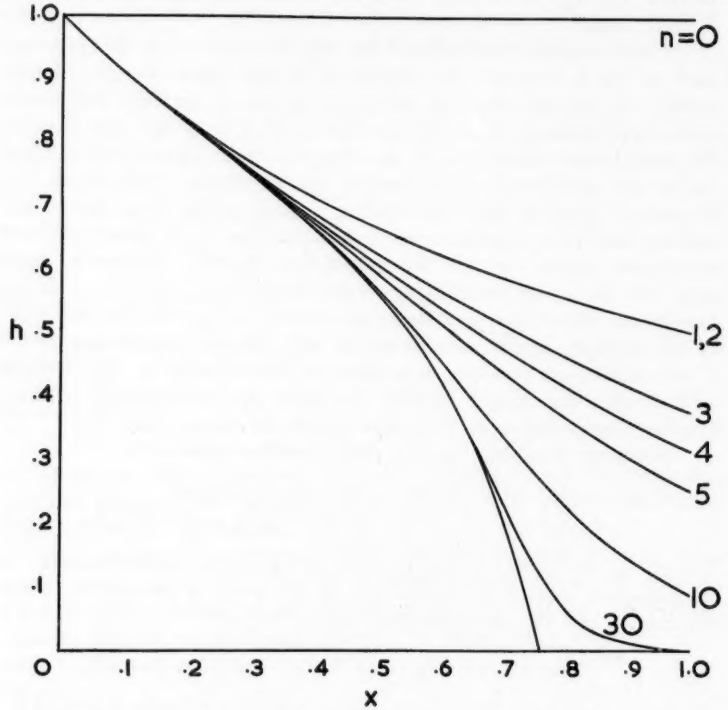


FIG. 2.—Approach to equilibrium under sib mating. h, x and n as in fig. 1.

maximum root is $x = 0{\cdot}7633$ (and therefore $d = 23{\cdot}7$). Below this value of x two-allele families form the stable state; above it homozygosis results. Fig. 2 depicts the approach to equilibrium. Near $x = 0{\cdot}76$ the rate of approach is very slow; even after 30 generations the population is still 12 per cent. heterozygous.

3.3. Parent-offspring mating.—Systems in which an individual is used twice, first as offspring and then as parent, will be discussed. This double use of an individual for two generations introduces certain complications: the survival rate, x, of the homozygotes compared with the heterozygotes may change over this period and the number of offspring may be insufficient to mate all the parents, or vice versa. We shall consider two cases, firstly where all parents are mated, and secondly where all of a given proportion of offspring are mated. The former needs a high birth-rate for low values of x, while in the latter the proportion mated must be low if the birth rate is high.

(i). All parents mated. Generally we may suppose that compared with the heterozygotes only x of the homozygotes survive to the first mating and y of these to the second mating. If $y = 1$ the population becomes homozygous for all x. If $y = x$ we have the following generation matrix where the parent is written first in the cross and we note that each mated couple must have at least one gene in common.

	$aa\times aa$	$aa\times ab$	$ab\times aa$	$ab\times ab$	$ab\times ac$
$aa\times aa$	x	.	x^2	.	.
$aa\times ab$	.	.	x	.	.
$ab\times aa$	.	x	.	x	x
$ab\times ab$	.	1	.	1	1
$ab\times ac$	.	.	.	.	2
Column divisors	1	$x+1$	$x+1$	$x+1$	$x+3$

The characteristic equation is

$$(\lambda-x)\{\lambda((x+1)^2\lambda^2-(x+1)\lambda-x^2)\}\{(x+3)\lambda-2\} = 0$$

As for sib-mating, homozygosis ensues near $x = 1$, while for low values of x two-allele families form the stable state. The changeover occurs at the maximum root of

$$x^3+2x^2-x-1 = 0 \qquad \textit{i.e.} \text{ at } x = 0{\cdot}8019.$$

(ii). All of a proportion of offspring mated. The generation matrix is

$$\begin{bmatrix} x & . & \frac{1}{2}x & . & . \\ . & . & \frac{1}{2} & . & . \\ . & \frac{1}{2}x & . & \frac{1}{2}x & \frac{1}{2}x \\ . & \frac{1}{2} & . & \frac{1}{2} & \frac{1}{2} \\ . & . & . & . & \frac{1}{2} \end{bmatrix}$$

The characteristic equation is

$$(\lambda - x)\{\lambda(4\lambda^2 - 2\lambda - x)\}(\lambda - \tfrac{1}{2}) = 0$$

and changeover from homozygous to mixed equilibrium occurs at $x = 0{\cdot}7500$.

3.4. Half-sib mating.—If a male taken at random is mated to all its half-sisters who are also half-sisters of one another, then the population may be classified into the groups of three which are the parents of each pair of half-sibs. The surplus males may be neglected. For simplicity only two alleles of the gene are considered in this case and even then there are 10 mating units.

The critical value of x is 0·8122, a root of an equation of the tenth degree.

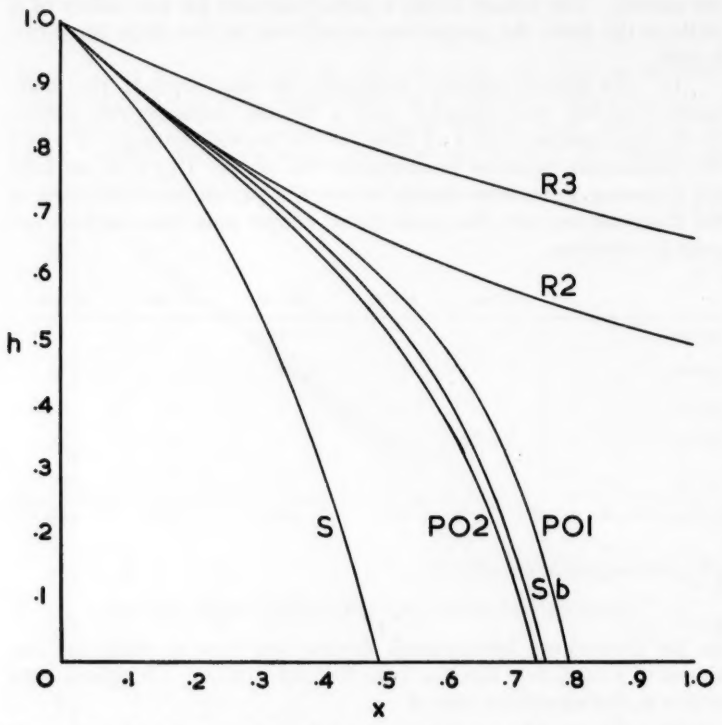


FIG. 3.—The relation of proportion of heterozygotes (h) to survival rate (x) at equilibrium under selfing (S), sib mating (Sb), two kinds of parent-offspring mating (PO1, PO2—see text), and random mating with two (R2) and three (R3) alleles.

3.5. Double first-cousin mating.—Again considering only two alleles, there are now 13 mating units, being all possible types of arrangement of four grandparents. The critical value of x is 0·8552.

3.6. Random mating.—With l alleles, the frequency of heterozygotes in the population $= h = \dfrac{l-1}{x+l-1}$ and each allele occurs equally often provided that $x \neq 1$.

Fig. 3 shows the frequency of heterozygotes in the stable state for selfing (S), sib (Sb) mating, parent-offspring (PO_1, PO_2) mating and random mating with two (R_2) or three (R_3) alleles.

4. TWO ALLELES WITH DIFFERENT VIABILITIES

In the foregoing all homozygotes were assumed to have equal disadvantages. Restricting ourselves to two alleles (a and b), we will now consider the case where the two homozygotes have unequal disadvantages, aa, bb, and ab having the respective viabilities x, y and 1 (x and $y \leqslant 1$). It obviously now becomes necessary to treat the two homozygotes separately in the generation matrix.

4.1. Selfing.—The generation matrix is

	aa	bb	ab
aa . .	x	.	$\frac{1}{4}x$
bb . .	.	y	$\frac{1}{4}y$
ab . .	.	.	$\frac{1}{2}$

The latent roots and corresponding equilibrium populations are

$= x$ $\quad V = (1\ ;\ 0\ ;\ 0)$

$= y$ $\quad V = (0\ ;\ 1\ ;\ 0)$

$= \frac{1}{2}$ $\quad V = (x(1-2y)\ ;\ y(1-2x)\ ;\ 2(1-2x)(1-2y))$

When $x > \frac{1}{2}, y$ the population becomes homozygous aa.

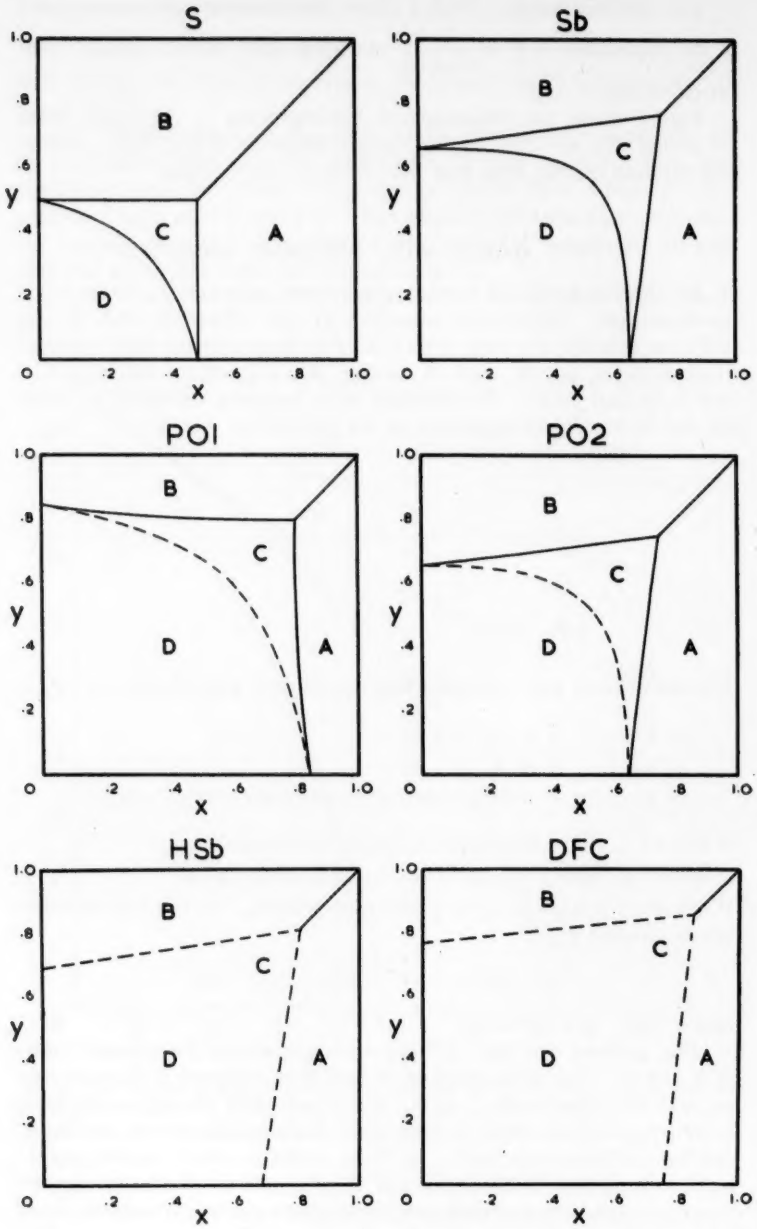
When $y > \frac{1}{2}, x$ the population becomes homozygous bb.

When $\frac{1}{2} > x, y$ some heterozygosity may remain. In this case Hardy's law is satisfied if

$$(1-2x)^2(1-2y)^2 = xy(1-2y)(1-2x)$$

i.e. $1-2x-2y+2xy = 0$.

Fig. 4 shows the type of final stable population for various values of x and y. The areas marked A and B correspond to homozygous aa and bb respectively; in C the population, though containing heterozygotes, has more homozygotes than a population mating at random without selection; in D it contains more heterozygotes; on the boundary between C and D, the effects of inbreeding and selection exactly counterbalance. There is a smooth transition across all boundary lines except that between A and B.


S
Sb
POI
PO2
HSb
DFC
A
B
C
D
X
Y
0
.2
.4
.6
.8
1.0

4.2. Sib mating.—The generation matrix is

	$aa\times aa$	$bb\times bb$	$aa\times ab$	$bb\times ab$	$ab\times ab$	$aa\times bb$
$aa\times aa$	x	.	$\frac{1}{2}x^2$	.	$\frac{1}{4}x^2$	.
$bb\times bb$	.	y	.	$\frac{1}{2}y^2$	$\frac{1}{4}y^2$	.
$aa\times ab$	.	.	x	.	x	.
$bb\times ab$	.	.	.	y	y	.
$ab\times ab$	.	.	$\frac{1}{2}$	$\frac{1}{2}$	1	1
$aa\times bb$	.	.	.	.	$\frac{1}{2}xy$	.
Column divisors	1	1	$x+1$	$y+1$	$x+y+2$	1

The characteristic equation is

$$(\lambda-x)(\lambda-y)f(\lambda, x, y) = 0$$

$f(\lambda, x, y)$ being a quartic in λ. Putting $\lambda = x$ in the quartic determines the boundary between A and C, and $\lambda = y$ the boundary between B and C. The Hardy law curve is given by an equation of the twelfth degree.

4.3. Parent-offspring mating.—Fig. 4 gives the regions for each type of equilibrium both for the case when all parents are mated (PO1) and when all of a proportion of the offspring are mated (PO2). The Hardy law curve has been dotted as it has been determined only at its endpoints and centre point.

Column (ii) of table 1 contains, for each inbreeding system, the values of d $(= 100(1-x))$ where $x = y$ cuts the Hardy law curve, *i.e.* at which the population is 50 per cent. heterozygous with equal proportions of each allele. These values may, of course, be obtained by solving $h=\frac{1}{2}$ in the formulæ of section 3.

Column (iii) of table 1 contains the value of d above which heterozygosity is possible when the other homozygote is lethal ($y = 0$).

4.4. Half-sib and double first-cousin mating.—These are too cumbersome for a full treatment, but the case when $y = 0$ has been investigated. The critical values of x are given in table 1.

4.5. Random mating.—The frequencies of *aa*, *bb* and *ab* are proportional to $x(1-y)^2$, $y(1-x)^2$, $2(1-x)(1-y)$ respectively. This agrees with the result given by Sturtevant and Mather (1938).

FIG. 4.—Phase diagrams showing the types of equilibrium achieved for two alleles, the homozygotes of which have different viabilities, under various systems of inbreeding (S, Sb, PO1, PO2, as in fig. 3, HSb = half-sib mating, DFC = double first-cousin mating).

x = survival rate of one homozygote (*aa*) compared with the heterozygote (*ab*).

y = survival rate of the other homozygote (*bb*).

When the point (x, y) is in A the population becomes homozygous *aa*, in B homozygous *bb*. In C the population becomes less heterozygous than a population mating at random without selection, in D more heterozygous.

4.6. The following results which emerge from this section are additional to those mentioned in sections 2 and 3.

(i) Strong selection against (even lethality of) one homozygote does not necessarily cause the corresponding allele to disappear from the population. Selection, not necessarily so strong against the other homozygote can hold the heterozygote in the population.

(ii) On the common boundaries of the areas A, B and C of fig. 4 two of the latent roots of the generation matrix are equal and at the common point of A, B and C three are equal. Near these boundaries the latent roots are nearly equal and two of them have a ratio equal to $1 : 1+\delta$ (where δ is very small) so that the relative contributions of the two corresponding latent vectors after n generations will be in the ratio $1 : (1+\delta)^n = 1+n\delta$, nearly, *i.e.* the latent vector corresponding to the smaller latent root disappears linearly and not exponentially. One example of this effect is in section 3 when, for $x = y$, it was noted that the disappearance of the heterozygote was very slow near the critical value of x. Another example was found in a more detailed investigation of mixed selfing and random mating, with general values of x and y, where the same phenomenon occurs near $x = y = 1$. This means that there is a critical region near the common boundaries of A, B and C in which, although the equilibrium is stable and fixed by the mating system and survival rate of the homozygotes, small disturbing influences may cause the population to drift widely from equilibrium.

5. OTHER MODES OF SELECTION

It has been assumed so far that there is no separation of the progeny of each mating, or at least, that this separation, if it exists, does not cause any difference between the relative survival rates of individuals in the same or in different families. This will be true of many plants in the wild or in populations of cultivated crops, but it may be only partly true of animals, depending on the stages in the animal's life cycle at which selection acts, or of experimental families of both plants and animals. We shall consider briefly two other schemes, one in which the same number of offspring is selected at random from each mating of a generation to provide matings for the next generation, and another in which it is assumed that all homozygotes having some heterozygous sibs show a survival rate differing from that of homozygotes all of whose sibs are homozygotes. Only the first three mating systems will be discussed in this section.

5.1. Constant number of progeny.—The generation matrices of sections 3 and 4 will now contain unity instead of x and y in their top left-hand corners. This is the maximum latent root of the matrix except at $x = 0$. Hence the population eventually becomes completely homozygous for all values of x.

5.2. Differential survival rates. Let z be the survival rate (compared with the heterozygotes) of homozygotes with only homozygous sibs,

and let x be the survival rate of the other homozygotes. The generation matrices are those of section 3 with the x in the top left-hand corner replaced by z. Then $\lambda_1 = z$, and λ_2 is, as before, the maximum latent root of the second sub-matrix down the leading diagonal. A graph of this maximum latent root against x shows, for any given x, the

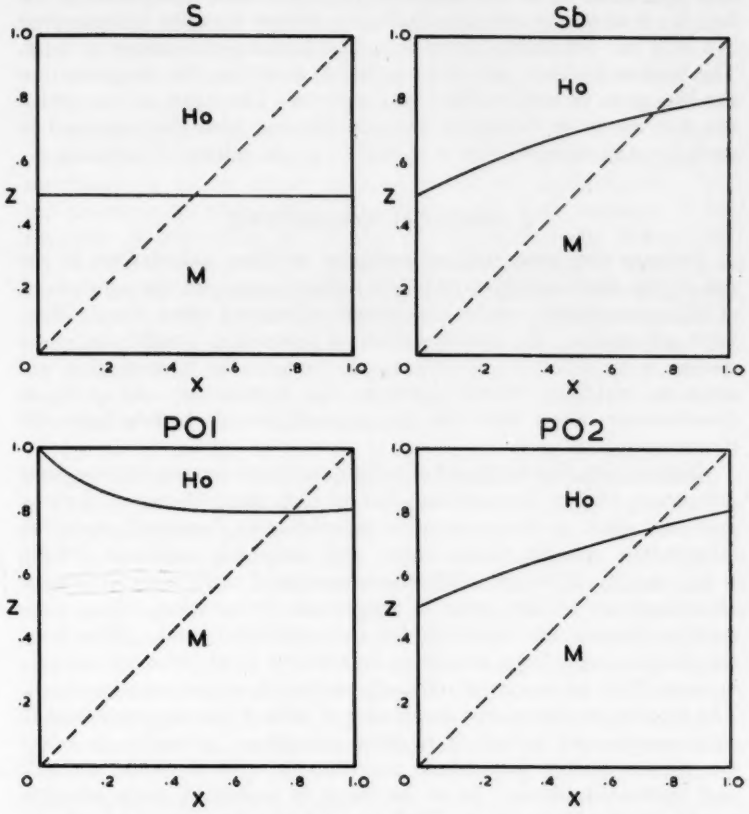


FIG. 5.—Phase diagrams showing the types of equilibrium when survival rates of homozygotes differ according to their origin.

z = survival rate of homozygotes with only homozygous sibs.

x = survival rate of the other homozygotes.

When the point (x, z) is in Ho the population becomes homozygous, in M some heterozygotes remain. Along the broken line $z = x$.

values which z must exceed for the population to become completely homozygous. Below this value some heterozygosity will always remain.

FIG. 5 shows phase diagrams for x and z for the three mating systems. The diagrams for half-sib mating and double first-cousin mating are

similar to those for Sb and PO_2. In the areas marked Ho the values of x and z are such that the population will eventually become homozygous ; in M it will remain mixed homozygous and heterozygous. As we expect, for low values of x, a value of z considerably greater than x is necessary for complete homozygosity, while for values of x near 1, z needs to be less than x to produce a mixed population. In fact, if z is near 1 no amount of selection against the other homozygotes can stop the population from becoming homozygous sooner or later. The broken line has only been included to divide the diagrams into the two parts in which either $x>z$ or $x<z$. The value of x at which this line meets the boundary between Ho and M is that obtained in section 3 and tabulated (as $d = 100(1-x)$) in column (i) of table 1.

6. PRACTICAL IMPLICATIONS

Perhaps the most striking outcome of these calculations is the size of the disadvantage which can suffice to prevent the attainment of full homozygosity under any system of mating other than selfing. With sib mating, the closest system of inbreeding possible in many species, a disadvantage of only 24 per cent. in the homozygotes will serve to maintain heterozygotes in the population, and even at disadvantages lower than this the progress towards homozygosis will be very slow.

Natural selection in favour of individuals heterozygous for structural differences of the chromosomes has already been shown to have a profound effect on the outcome of inbreeding in *Campanula persicifolia* (Darlington and La Cour, 1950) and *Drosophila subobscura* (Philip *et al.*, 1944). Evidently differences associated with these structural alterations are of the order of magnitude in question. Few comparative figures are available for uncomplicated genic differences, but disadvantages large enough to be effective seem to be not unlikely in most if not all species of naturally outbreeding plants and animals. The relative weakness and incapacity of inbred lines is proverbial in such species, and indeed it is often considered desirable to select for vigour during inbreeding programmes with domestic animals and cultivated plants. In so far as it is successful, such selection would, it would seem, be likely to achieve its object largely by delaying or even in some circumstances preventing progress of the inbreeding.

The vitiating effects on the progress of inbreeding of the disadvantage, which homozygotes may suffer, may of course be reduced by suitable management in experiment. Any step which reduces the intensity of competition between individuals even within families should assist towards this end. In plants, the avoidance of crowding in seed pans, in seedling boxes and in the field, will help to reduce the intensity of such competition. The individuals to be taken for breeding can also be chosen without reference to their vigour or

fertility so that their representatives in the next generation will be independent of their phenotypes. Even so, however, there must always remain the possibility of competition between pollen grains and tubes in the process of fertilisation, and there is the further possibility of competition between developing embryos, especially where many seeds are normally developed in the capsule. It is thus likely that although the effects of disadvantage on the attainment of homozygosity may be reduced, they will not be eliminated entirely.

In the same way intra-uterine competition and post-natal competition during suckling will always be potential sources of disturbance in mammals, as will post-hatching competition in birds or larval competition in insects, except where each individual is raised virtually separately—a course which must in general be impracticable. It is not therefore surprising that Dzügünes (1950) finds evidence of slow progress of inbreeding in poultry. Progress towards homozygosis may be aided even more by ensuring that the offspring of homozygous parents are not at a disadvantage with respect to those of heterozygotes, that is by ensuring that, so far as possible, all pairs of partners contribute equally to the next generation. This precaution may, however, only be partially successful since even in the absence of competition from heterozygotes the homozygotes may die out differentially by reason of reduced vigour and disease resistance.

In so far as the fitness of homozygotes depends on polygenic systems the present findings must be applied with caution, since it is characteristic of such systems that new and superior balances can be achieved given sufficient time. This is, however, more of a long term than a short term possibility. The linked polygenic combinations will be expected to behave more or less as units, effective factors as they have been called (Mather, 1949), for short periods, their properties of change and readjustment becoming important only as the generations accumulate. The effects of disadvantage of homozygosis for polygenic combinations may be expected to be felt with full force in the average inbreeding experiment, even though this disadvantage can be mitigated in the long run by readjustments such as must have occurred during the rise of inbreeding species from outbreeding ancestors in nature (Darlington and Mather, 1949).

In designing inbreeding programmes, especially in outbreeding species, the consequences of disadvantage of homozygotes must be faced. Two implications of such disadvantage are clear. First, the inbreeding should be as close as possible: there is nothing to be gained from slow inbreeding. If the lines produced by close mating are too poor to survive or to be of value, anything better which results from a looser mating system will be better only because it has stabilised at a higher level of heterozygosity. Such a line may be of value where an equilibrium at which lower homozygosity is combined with a reasonable fitness is preferable to high homozygosity accompanied by low fitness, as may well be the case in crop and stock

improvement work. But let such a situation be recognised for what it is, and any delusion dispelled that the same level of homozygosity has been produced by the slow inbreeding, albeit at the sacrifice of time, as would have been achieved by a closer mating system.

The second point concerns the interruption of a system of inbreeding. In the absence of selective disadvantage, the interruption of a system by the substitution of a looser mating for a generation or so will in general merely slow down the progress towards homozygosity (Fisher, 1949). When the homozygotes are at a disadvantage, however, an actual loss of homozygosity might occur. Such will be the case when the proportion of homozygotes, though lower than that at which equilibrium would be reached with the closer system of inbreeding, is higher than that characteristic of the looser mating type. Since these various equilibria are all stable, the proportion of homozygotes will fall back towards the equilibrium value characteristic of the looser system should it have become too high by reason of the closer systems applied earlier. The same will be true when the proportion has risen too high as a result of sampling variation, except, of course, where the drift has led to complete homozygosis in all the breeding lines. In such a case only mutation could lead to a reapproach to the normal equilibrium.

Drift may have another effect. Where the two alternative homozygotes are equally, or nearly equally, fit, they will, apart from drift, tend to appear in the population with frequencies relative to one another which reflect the initial gene frequencies of the outbred ancestors rather than the relative fitness. The relative frequencies of the homozygotes would in such a case be liable to marked change by drift in small populations. Where, however, the two homozygotes differ sharply in fitness, their relative frequencies will be determined chiefly by this difference and selection will correct any changes which may arise from drift.

One last point remains to be made. Agreement of the proportions of the two homozygotes and the heterozygote in a population with the Hardy law is apt to be taken as indicating that mating is at random within that population. Certainly, such an agreement will follow from random mating, other things being equal. We can now see, however, that the Hardy proportions may appear even with close inbreeding, provided the homozygotes are at the necessary disadvantage, and this disadvantage, though high in a system of complete selfing, is not impossibly great with looser inbreeding (table 1 (ii)). Indeed disadvantages of a magnitude which experiment would suggest as not impossible in a natural population, may combine with a moderately inbreeding system to give proportions of homozygotes lower than expected from Hardy's series. Balanced polymorphism may therefore accompany inbreeding. The extreme case of this is, of course, found in *Oenothera* where regular selfing is accompanied by permanent hybridity because homozygotes are either lethal or

are prevented from occurring by special kinds of gametic lethality and competition. Given a knowledge of the selective properties of the various genotypes, conclusions may be drawn about the system of mating prevailing in a population from the frequencies of the genotypes. But in the absence of such information, neither random mating nor any other system can be inferred with full confidence.

7. SUMMARY

The effect of selection against homozygotes on the progress of inbreeding in large populations is considered. At disadvantages above a critical value which varies with the system of inbreeding (see table 1), complete homozygosis is never obtained, the population continuing to carry heterozygotes at equilibrium.

Diagrams are given to show the progress of inbreeding and the equilibrium reached for the case where any number of alleles are present, but all homozygotes have the same relative disadvantage, and for the case of two alleles, the homozygotes for which may have different disadvantages.

Consideration is extended to situations in which the homozygous offspring of homozygous parents are at a lesser disadvantage than homozygotes at least one of whose parents was heterozygous. Where all parents contribute equally to the next generation, the final state of the population under the inbreeding systems discussed will be one of homozygosity.

The practical implications of these findings are discussed and it is emphasised that with homozygotes at a disadvantage experimental inbreeding need not lead to homozygosis ; that inbreeding should be as close as possible if homozygosis is being sought ; that interruption of the inbreeding may cause loss of homozygosis ; and that agreement with the Hardy law need not imply random mating.

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MIXED SELFING AND RANDOM MATING WHEN HOMOZYGOTES ARE AT A DISADVANTAGE

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1. INTRODUCTION

THIS investigation arose as part of a general investigation (Hayman and Mather, 1953) of behaviour under inbreeding when homozygotes are at a selective disadvantage compared with heterozygotes. The inbreeding systems there discussed were handled by matrix methods not applicable to mixed selfing and random mating, for which an elementary algebraic treatment is necessary. This mating system occurs in plants which have an imperfect selfing mechanism combined with either widespread pollen dispersal or seed mixing by an external agency (as in cultivated crops).

One gene with only two alleles, a and b, is considered, so that a population containing this gene may be classified into the three genotypes aa, bb, and ab with frequencies p, q and r. Any such population may be represented by a point in a trilinear diagram, based on an equilateral reference triangle, and such that the lengths of the perpendiculars from the " population point " to the sides of the triangle are proportional to p, q and r. We shall investigate how a large population changes from generation to generation under certain conditions, and represent these changes by curves in a trilinear diagram. Further, we shall obtain phase diagrams, similar to fig. 4 of the paper quoted, showing broadly the equilibrium populations for various magnitudes of the selective disadvantages of the homozygotes aa and bb. The populations are assumed to be large enough for random fluctuations in gene frequencies to be unimportant.

2. EQUILIBRIUM

Let p, q and r ($p+q+r = 1$) be the frequencies of aa, bb and ab respectively and let x, y and 1 (x and $y \leqslant 1$) be their respective viabilities. Let s and t ($s+t = 1$) be the frequencies of selfing and random mating.

Starting with a population (p_0, q_0, r_0) and no selection, the frequencies in the next generation under selfing are

$$\{p_0+\tfrac{1}{4}r_0,\ q_0+\tfrac{1}{4}r_0,\ \tfrac{1}{2}r_0\}$$

while under random mating they are

$$\{(p_0+\tfrac{1}{2}r_0)^2,\ (q_0+\tfrac{1}{2}r_0)^2,\ 2(p_0+\tfrac{1}{2}r_0)(q_0+\tfrac{1}{2}r_0)\}$$

Under the mixed system and in the presence of selection they become (p_1, q_1, r_1) where

$$\left.\begin{aligned} p_1 &\propto x\{s(p_0+\tfrac{1}{4}r_0)+t(p_0+\tfrac{1}{2}r_0)^2\} = x\{p_0+\tfrac{1}{4}(sr_0-te_0)\} \\ q_1 &\propto y\{s(q_0+\tfrac{1}{4}r_0)+t(q_0+\tfrac{1}{2}r_0)^2\} = y\{q_0+\tfrac{1}{4}(sr_0-te_0)\} \\ r_1 &\propto \tfrac{1}{2}sr_0+2t(p_0+\tfrac{1}{2}r_0)(q_0+\tfrac{1}{2}r_0) = r_0-\tfrac{1}{2}(sr_0-te_0) \end{aligned}\right\} \quad . \quad (1)$$

$$\text{and} \qquad e = 4pq-r^2$$

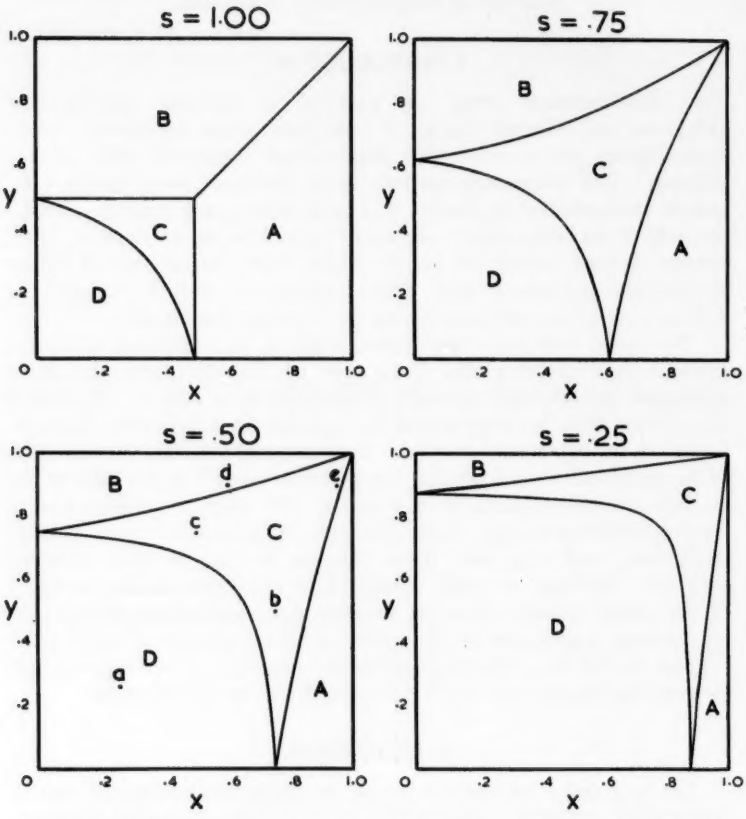

FIG. 1.—Phase diagram showing types of equilibrium with various proportions of selfing (s) and random mating ($t = 1-s$).

x = survival rate of one homozygote (aa) relative to that of the heterozygote (ab).

y = relative survival rate of the other homozygote (bb).

When the point (x, y) is in A, the population becomes homozygous aa, in B, homozygous bb. In C, the population becomes less heterozygous than a population mating at random without selection, in D, more heterozygous.

The points a, b, c, d and e in the diagram for $s = 0{\cdot}50$ are those referred to in *4.2* and fig. 4.

The population is in equilibrium if $\frac{p_1}{p_0} = \frac{q_1}{q_0} = \frac{r_1}{r_0}$, *i.e.* when

$$x\{1+\frac{1}{4p}(sr-te)\} = y\{1+\frac{1}{4q}(sr-te)\} = 1-\frac{1}{2r}(sr-te) \quad . \quad (2)$$

These equations determine p, q and r in terms of x, y and s.

Fig. 1 shows the type of equilibrium population for various values of x, y and s. The areas marked A and B correspond to homozygons *aa* and *bb* respectively; in C the population, though containing heterozygotes, has more homozygotes than a population mating purely at random without selection; in D it contains more heterozygotes; on the boundary between C and D, the effects of inbreeding and selection exactly counterbalance each other. (The small letters in the diagram for $s = \frac{1}{2}$ will be referred to in *4.2*.)

The inner boundaries of A and B are obtained by making r tend to zero in the equilibrium equations (2). The boundary between A and C is

$$2sxy-2x^2+2x-sx-sy = 0.$$

Interchanging x and y gives the boundary between B and C.

Hardy's law is the statement that $e = 0$. Eliminating p, q and r from this and equations (2) produces

$$(2+s)xy-2x-2y+2-s = 0.$$

This is the boundary between C and D. All three of these curves are hyperbolas.

A point to note is that strong selection against (even lethality of) one homozygote does not necessarily cause the corresponding allele to disappear from the population. Selection against the other homozygote can hold the heterozygote in the population.

3. EXACT SOLUTIONS

3.1. When $x = y = 1$, equations (2) show that the equilibrium state satisfies $sr-te = 0$. This is a parabola in the (p, q, r) trilinear diagram passing through the lower vertices of the reference triangle. Fig. 2 contains equilibrium parabolas for $s = 0, \frac{1}{2}, \frac{3}{4}$ and 1.

3.2. When $x = y < 1$, then from (2) $p = q$, and therefore

$$e = (2p-r)(2p+r) = 2p-r.$$

Eliminating p, q and t from (2) in this case leaves

$$xr(1+s-sr) = (1-r)(1-s+sr),$$

giving the frequency, r, of the heterozygote in terms of the survival rate, x, of the homozygotes. Graphs of r against x for $s = 0, \frac{1}{4}, \frac{1}{2}, \frac{3}{4}$ and 1 are shown in fig. 3.

In the trilinear diagram the equilibrium point is

$$(\tfrac{1}{2}(1-r), \tfrac{1}{2}(1-r), r)$$

on the perpendicular bisector of the base of the reference triangle.

3.3. General values of x and y. Eliminating $sr-te$ from equations (2) leaves

$$aq(r+2p) = p(r+2q) \quad . \quad . \quad . \quad . \quad (3)$$

where $$ay(1-x) = x(1-y).$$

Equations (3) are, for various values of a, a set of hyperbolas through the vertices of the reference triangle.

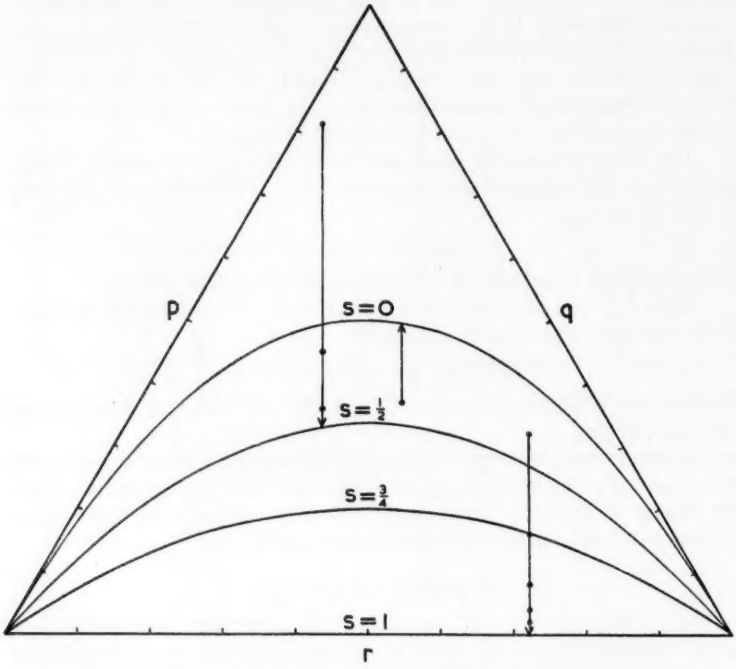

FIG. 2.—Trilinear diagram showing approach to equilibrium in the absence of selection. Distances from the sides labelled p, q and r represent the proportions of aa, bb and ab respectively in the population. s is the proportion of selfing.

The points of the parabolas represent possible equilibrium populations for the corresponding values of s. The approach to equilibrium is shown for three cases by arrowed lines, the dots indicating the position of the population in successive generations.

Now there will be an equilibrium parabola through each equilibrium point. If it has equation

$$(1-\beta)r-\beta e = 0 \quad . \quad . \quad . \quad . \quad (4)$$

it must be satisfied by the solution of (2). The value of β for this to be so can be shown to be the positive root of

$$\beta^2(2xy-x-y)+\beta\{(4-2t)xy-(3-t)(x+y)+2\}+t(2-x-y) = 0$$

Once α and β have been evaluated, we can solve (3) and (4) for the equilibrium point. The solutions are

	C, D	A	B
$p =$	$d\alpha\{\beta(1+\alpha)-1+\alpha\}$	1	0
$q =$	$d\{\beta(1+\alpha)+1-\alpha\}$	0	1
$r =$	$d\{\beta^2(1+\alpha)^2-(1-\alpha)^2\}$	0	0

with $d^{-1} = \beta(1+\beta)(1+\alpha)^2$. The appropriate solution is that corresponding to the area (A, B, C or D) in which (x, y) lies in fig. 1. When

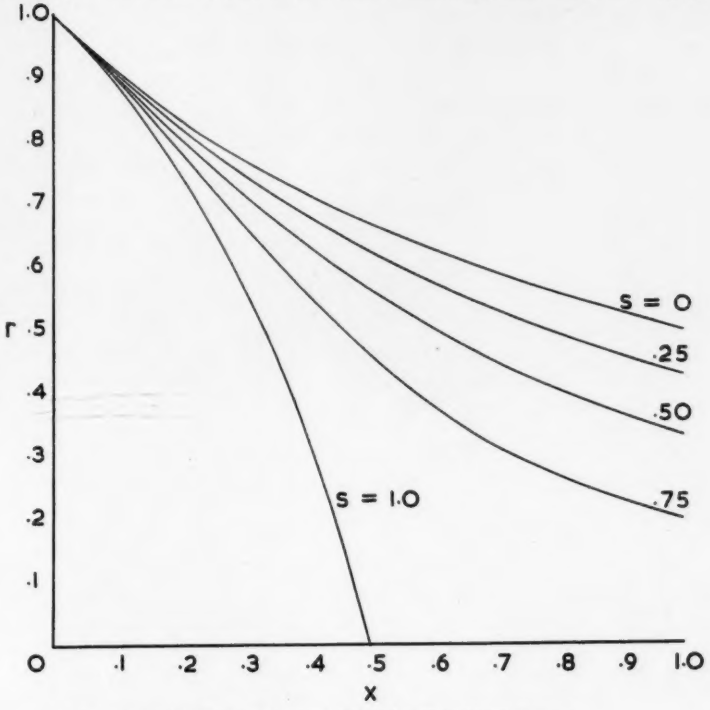

FIG. 3.—Equilibrium with equal viabilities of the homozygotes.
r = proportion of heterozygotes in the population.
x = survival rate of both homozygotes compared with the heterozygote.
s = proportion of selfing.

s is given and (x, y) lies in C or D the equilibrium point lies above the parabola $sr - te = 0$, since the population must eventually become more heterozygous than it would in the absence of selection against the homozygotes.

Evidently when x and y are less than unity, equilibrium is independent of the initial state (unless one allele is initially absent) and is determined by the two parameters α and β. As these are functions of the three population parameters x, y and s, it is clear that different sets of values of x, y and s can determine the same pair of values of α and β, and hence the same equilibrium. This means, for example, that an increase in the proportion of selfing can be exactly counterbalanced by a decrease in the viability of the homozygotes. The diagrams of fig. 1 are sections for given s of a 3-dimensional graph of x, y and s, and in this graph each line of intersection of a pair of surfaces, given by equations (3) and (4) determines one equilibrium point.

4. THE APPROACH TO EQUILIBRIUM

4.1. When $x = y = 1$, the statements (1) lead to the well-known results for the case of no selection. The proportionalities now become equations and further $sr_1 - te_1 = \frac{1}{2}s(sr_0 - te_0)$

$$\begin{aligned} \therefore p_n &= p_0 + \tfrac{1}{2}m_n \to p_0 + \tfrac{1}{2}m = u^2 + suv/(2-s) \\ q_n &= q_0 + \tfrac{1}{2}m_n \to q_0 + \tfrac{1}{2}m = v^2 + suv/(2-s) \\ r_n &= r_0 - m_n \to r_0 - m \quad = 2uv(2-2s)/(2-s) \end{aligned}$$

where $$m_n = (sr_0 - te_0)(1 - (\tfrac{1}{2}s)^n)/(2-s)$$

and $$m = (sr_0 - te_0)/(2-s)$$

m determines the limiting frequencies at equilibrium. The second form for the limiting frequencies indicates departures from a random mating population since $u = p_0 + \frac{1}{2}r_0$ and $v = q_0 + \frac{1}{2}r_0$, which are the gene frequencies.

In 3.1 it was shown that the equilibrium point lies on the parabola $sr - te = 0$ in the trilinear diagram. Now $p_n + \frac{1}{2}r_n = p_0 + \frac{1}{2}r_0$ and $q_n + \frac{1}{2}r_n = q_0 + \frac{1}{2}r_0$, which also follow directly from the constancy of gene frequencies (assuming no mutation). Hence the point (p_n, q_n, r_n) lies on the perpendicular line from (p_0, q_0, r_0) to the base of the reference triangle. Evidently as n increases the population point proceeds along this line until it reaches the equilibrium parabola, the distance from equilibrium being reduced to $\frac{1}{2}s$ of its previous value every generation. Fig. 2 illustrates three cases when $s = 0$, $\frac{1}{2}$ and 1, the first attaining equilibrium in one generation. The dots indicate successive population points.

4.2. For general values of x and y, successive applications of the proportionalities (1) enable the population point to be plotted from generation to generation, while the limit point may be determined independently by 3.3. Fig. 4 illustrates the approaches to equilibrium starting with two different populations F = (0·6, 0·3, 0·1) or G = (0·1, 0·3, 0·6) under an equal mixture of selfing and random mating ($s = \frac{1}{2}$). Commencing at F, the first few and some subsequent

generations have been indicated by numbered dots. Five sets of selection values (x, y) giving five equilibrium points have been chosen as in the table.

	Equilibrium points				
	a	*b*	*c*	*d*	*e*
Selection values x . .	0·259	0·750	0·500	0·600	0·950
y . .	0·259	0·500	0·750	0·900	0·900
Equilibrium frequencies p .	0·125	0·634	0·057	0·000	0·724
q .	0·125	0·057	0·634	1·000	0·072
r .	0·750	0·309	0·309	0·000	0·204

These have also been labelled in fig. 1 for $s = \frac{1}{2}$.

Some points may be noted. Most of the initial disequilibrium

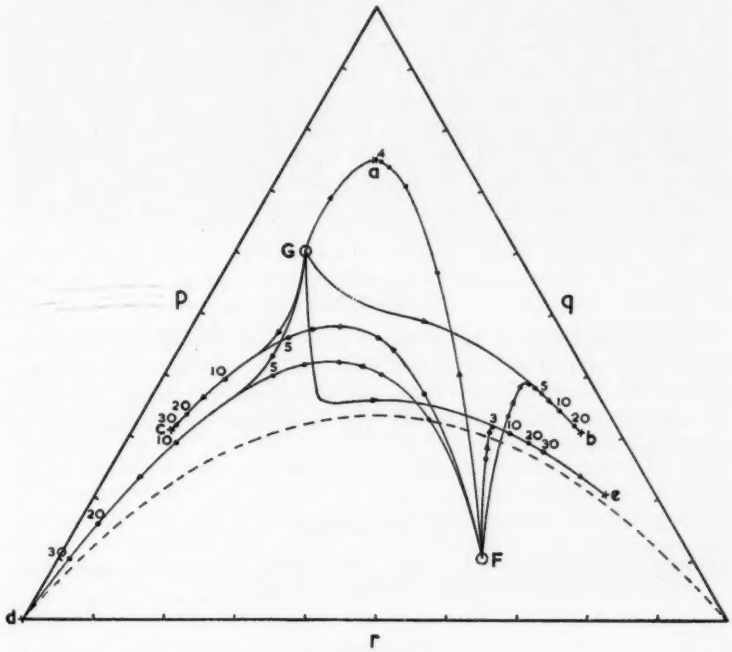

FIG. 4.—Trilinear diagram of the approach to equilibrium when the proportion of selfing, $s = \frac{1}{2}$.

F and G are two possible starting points while *a*, *b*, *c*, *d*, and *e* are five equilibrium points corresponding to the values of (x, y) shown in fig. 1, $s = \frac{1}{2}$. The solid lines show the paths of the population from F or G to *a*, *b*, *c*, *d* or *e*. The dots on the lines from F show the position of the population after the number of generations indicated.

The broken line represents possible equilibria in the absence of selection.

between heterozygotes and homozygotes is rapidly removed: the disequilibrium between the two homozygotes disappears very slowly.

With strong selection against the homozygotes the equilibrium is attained quickly and fairly directly. With weak selection against the homozygotes, the equilibrium parabola through the equilibrium point is first approached quickly and nearly vertically, thus correcting any heterozygote-homozygote disequilibrium. Then the population point moves slowly round close to this parabola to the final equilibrium point, thus correcting any disequilibrium between the two homozygotes.

We recall that when there is no selection against the homozygotes the equilibrium depends on the initial gene frequencies. Hence, if fluctuations in these frequencies occur (by chance or by mutation) the equilibrium point shifts around the equilibrium parabola (determined by s) since there is no controlling force to hold it at any particular point on the parabola.

When there is only weak selection against the homozygotes a similar situation prevails. In case (e) and starting from (0·6, 0·3, 0·1) the population point is still distant 0·125 units from the equilibrium point after 30 generations and the next generation only reduces this gap by 2·1 per cent. This slow approach to equilibrium shows that the force working towards equilibrium is weak. This means that, though in general the equilibrium is stable and is fixed by the mating system and the survival rate of the homozygotes, there is a region near $x = y = 1$ in fig. 1 in which small disturbing influences may cause the population point to drift widely away from equilibrium along the equilibrium parabola.

5. SUMMARY

The equilibrium is investigated of a large population breeding under a system of mixed selfing and random mating with selection against the homozygotes. Diagrams are produced giving the types of equilibrium and illustrating the approach to equilibrium in particular cases.

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A COMPARATIVE STUDY OF CHROMOSOMAL POLYMORPHISM IN CERTAIN SOUTH AMERICAN SPECIES OF *DROSOPHILA*

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1. INTRODUCTION

NATURAL populations of most species of Drosophila are variable with respect to the gene arrangement in the chromosomes of different individuals. In some species, a considerable proportion of flies found in nature are inversion heterozygotes. The degree of the variability of the chromosome structure varies however in geographic races of the same species, as well as in different species. To account for the different variability of different races and species, da Cunha, Burla and Dobzhanksy (1950), and Dobzhansky, Burla and da Cunha (1950) have advanced the following working hypothesis. Since the chromosomal polymorphism in Drosophila populations is adaptive and balanced, the amount of this polymorphism present in a race or a species is likely to be a function of the number and variety of the ecological niches which the population has mastered. Other things being equal, species which exploit many different kinds of food, or which occur in many different biotopes, are likely to exhibit a greater degree of adaptive polymorphism than do species which are specialised to exploit only a limited variety of adaptive opportunities.

The data reported in the present paper have been collected in order to submit the above working hypothesis to further tests. We have chosen several species of Drosophila which are favourable for studies on the chromosomes in the larvæ salivary glands. The amounts of the chromosomal variability were then compared either in pairs of closely related species, or in different populations of the same species. The data so obtained can be evaluated against the background of the ecological characteristics of the respective races and species described by Dobzhansky and Pavan (1950), Pavan (unpublished), and Cordeiro (unpublished).

2. METHODS

Single females collected in nature were allowed to produce offspring in individual cultures with banana-agar nutrient medium. The salivary glands of mature larvæ produced in the cultures were stained with acetic orcein. The chromosomes of a single larva in the offspring of each wild progenitor were then examined.

3. *DROSOPHILA POLYMORPHA* AND *DROSOPHILA CARDINOIDES*

D. polymorpha Dobzhansky and Pavan and *D. cardinoides* Dobzhansky and Pavan are closely related species belonging to the *cardini* species group of the Subgenus Drosophila. They are sympatric in a large area, which extends, as far as known, from northern Argentina to Central America. The metaphase chromosome groups of both species are alike; they contain two pairs of metacentric autosomes (the second and the third), a pair of acrocentric X-chromosomes, and a pair of dot-like autosomes. The inversions found in these chromosomes are described by da Cunha and Salzano (in press).

TABLE 1

Frequencies (in per cent.) of various inversions found in heterozygous condition, and the mean numbers of heterozygous inversions per individual in D. polymorpha

Chromosome	Inversions	Vila Atlantica	Pirassununga	Mogí das Cruzes
II	none	71·6	98·8	98·7
	Aa	28·4	1·2	1·3
III	none	92·5	100	100
	Aa	7·5	...	...
	Bb	7·5	...	...
X	none	43·3	63·6	82·5
	Aa	43·3	36·4	17·5
	Bb	56·7	36·4	17·5
	Cc	43·3	...	17·5
Mean per individual	♂	*0·46±0·08*	*0·01*	*0·01*
	♀	*1·82±0·18*	*1·10±0·16*	*0·54±0·14*
Flies tested	♂	67	83	93
	♀	67	88	63

The chromosomal polymorphism in *D. polymorpha* is greater than that in *D. cardinoides*. In the former species, six different inversions have been found in 461 wild individuals collected in three localities, while in *D. cardinoides* three inversions were found in 141 flies, also from three localities. More important are the data on the frequencies of heterozygosis for inversions in the populations of the two species, summarised in tables 1 and 2.

Sufficient samples of *D. polymorpha* were available from three localities in the State of São Paulo, Brazil, namely Vila Atlantica, Pirassununga, and Mogí das Cruzes. The mean numbers of heterozygous inversions, per female, were 1·82, 1·10 and 0·54 in the three localities. The mean numbers in the males are lower, since no

inversion heterozygosis can be detected in the X-chromosome in the males.

Samples of *D. cardinoides* were studied from Vila Atlantica (State of São Paulo), from Cantareira (same State, about 50 km. from Mogí das Cruzes), and from Catuní (State of Bahia). In all these samples the inversions proved to be rare. The numbers of heterozygous inversions per individual were 0·06, 0 and 0·05 in the three localities respectively.

D. polymorpha is a species which is relatively more common, at least in southern Brazil, than *D. cardinoides*. At Pirassununga, the frequencies of *D. polymorpha* vary from 0·2 to 22 per cent. of the total catch of all species of Drosophila, depending upon season. *D. cardinoides* is much less frequent, varying from 0·03 to 0·5 per cent.

TABLE 2

Frequencies (in per cent.) of various inversions found in heterozygous condition, and the mean numbers of heterozygous inversions per individual in D. cardinoides

Chromosome	Inversions	Bragança	Cantareira	Vila Atlantica	Catuni
II	none	83	100	100	94
	Aa	17	...	...	...
	Bb	...	...	...	6
III	none	100	100	93	100
	Aa	...	...	7	...
Mean per individual . .		*0·16*	*0*	*0·06±0·04*	*0·05±0·03*
Flies studied . . .		6	50	46	55

of the total catch. At Mogí das Cruzes, *D. polymorpha* varies from 0·9 to 22 per cent., and *D. cardinoides* from 0·01 to 3·5 per cent. of all Drosophila. At Vila Atlantica, from 0·2 to 3 per cent. of the flies are *D. polymorpha* and 0·1 to 4·3 per cent. are *D. cardinoides*. It should be noted that the high proportions of *D. cardinoides* have been observed close to human habitations, and the proportions in undisturbed localities are always below 1 per cent. Similarly, at Cantareira and at Catuní, *D. cardinoides* has been collected only in orchards, near human habitations. *D. polymorpha* is a species which we have not collected under such conditions.

4. *DROSOPHILA GUARAMUNU* AND *DROSOPHILA GRISEOLINEATA*

D. guaramunu Dobzhansky and Pavan and *D. griseolineata* Duda are closely related to each other, and both of them belong to the *guarani* species group of the subgenus Drosophila (King, 1947). Their known distribution regions extend from northern Argentine to central

Brazil. In both species, the metaphase chromosome plates contain five pairs of rod-shaped and one pair of dot-like chromosomes. A total of 16 kinds of inversions have been found in the offspring of 896 wild individuals of *D. guaramunu* from seven different localities. A description of these inversions can be found in the paper of Brncic (in press). In *D. griseolineata*, 5 kinds of inversions have been encountered in the offspring of 612 wild flies from six localities. These inversions are to be described by Salzano (unpublished).

TABLE 3

Frequencies (in per cent.) of various inversions in heterozygous condition, and the mean numbers of heterozygous inversion per individual of D. guaramunu

Chromosome	Inversions	Chapada	Ponta Grossa	Itapoan	Feliz	Bragança	Mogí das Cruzes	Pirassununga
II	None	60·8	56·4	60·0	48·5	45·0	50·8	47·5
	Aa	39·2	43·6	40·0	51·5	55·0	49·2	52·5
III	None	43·4	41·8	50·0	51·5	45·0	49·2	47·9
	Aa or *Bb*	56·6	47·1	50·0	41·0	50·0	43·4	47·9
	Aa Bb	...	10·9	...	7·5	5·0	7·4	4·1
IV	None	32·6	74·5	30·0	30·3	30·0	49·2	40·1
	Aa	19·5	3·6	10·0	21·2	25·0	25·4	20·6
	Bb	15·1	10·9	...	18·1	25·0	19·7	15·2
	Cc	...	...	...	...	...	...	0·2
	Dd	1·1	...	...	4·5	5·0	0·8	2·4
	Ee	15·1	14·5	30·0	36·2	30·0	0·8	13·9
	Ff	...	...	...	...	...	...	0·4
	Gg	4·3	3·6	...	1·5	10·0	6·5	3·7
	Hh	14·1	14·5	30·0	31·8	30·0	0·8	12·6
	Ii	5·4	...	...	...	20·0	1·7	5·6
	Jj	5·4	...	...	...	20·0	1·7	5·6
	Kk	5·4	...	...	...	20·0	1·7	5·6
	Ll	5·4	...	...	...	20·0	1·7	5·6
	Mm	...	...	...	...	...	1·7	...
Mean per individual		*2·13±0·13*	*1·65±0·14*	*1·60±0·38*	*2·18±0·17*	*3·20±0·46*	*1·66±0·09*	*2·00±0·06*
Flies tested		92	55	10	66	20	192	461

The frequencies of the various inversions in the population samples studied are summarised in tables 3 and 4. It can be seen at a glance that the chromosomal variability is much greater in *D. guaramunu* than in *D. griseolineata*. In the former species, the localities in the State of Rio Grande do Sul (Chapada, Ponta Grossa, Itapoan and Feliz) have between 1·6 and 2·2 inversions per individual. In the State of São Paulo (Bragança, Mogí das Cruzes, Pirassununga) the inversion frequencies are about the same as in Rio Grande do Sul (1·7 to 3·2, the latter figure being subject to a large error). Since no inversions have been detected in the X-chromosome of these

species, the data for females and males can be combined. In *D. griseolineata*, the State of Rio Grande do Sul (Ponta Grossa, Itapoan, Emboaba) shows perhaps fewer inversions per individual than does the State of São Paulo (Vila Atlantica, Cantareira, and Mogí das Cruzes). But even the highest recorded inversion frequency per individual of *D. griseolineata* (0·47, *cf.* table 4) is lower than the lowest frequency found in *D. guaramunu* (1·6, *cf.* table 3).

According to Dobzhansky and Pavan (1950), *D. guaramunu* is more common and widespread in Brazil than is *D. griseolineata*. The former species proved to be one of the commonest species of Drosophila in 5 out of the 35 localities studied by these authors. The latter species

TABLE 4

Frequencies (in per cent.) of various inversions found in heterozygous condition, and the mean numbers of heterozygous inversion per individual of D. griseolineata

Chromosome	Inversions	Ponta Grossa	Itapoan	Emboaba	Vila Atlantica	Cantareira	Mogí das Cruzes
II	none	96·5	97·1	98·6	80·8	90·6	82·9
	Aa	...	...	...	1·1	...	2·6
	Bb	3·5	2·9	1·4	14·0	8·0	9·2
	Cc	...	...	...	4·1	1·4	5·3
III	none	94·7	100·0	100·0	86·4	81·0	86·8
	Aa	5·3	...	...	13·6	19·0	14·0
	Bb	5·3	...	...	13·2	19·0	14·0
Mean per individual		*0·14±0·06*	*0·02±0·06*	*0·01±0·04*	*0·47±0·04*	*0·47±0·09*	*0·46±0·09*
Flies tested . .		57	69	71	265	74	76

has been found in 6 out of the 35 localities, but in all of them it was one of the rarer forms. *D. guaramunu* is, indeed, one of the dominant species both in the savanna of central Brazil and in the subtropical forest of the State of Rio Grande do Sul. At Pirassununga, which represents ecologically a southern outlier of the savanna of central Brazil, the lowest frequency of *D. guaramunu* in 18 different collections made during four years of observation was about 2 per cent., and in 9 of these collections it was above 18 per cent., of the total catch of Drosophila flies. At Mogí das Cruzes, its frequency varied from 0 to 23 per cent. of the total, and at Vila Atlantica from 0 to 6 per cent. of the total. In Rio Grande do Sul, *D. guaramunu* is encountered with frequencies from 0·5 to 5 per cent. on the coast, and from 11 to 51 per cent. in the interior of the State.

D. griseolineata varies greatly in frequency, but it is on the whole much less common than *D. guaramunu*. The recorded frequencies of the former species vary from 0 to 30 per cent. at Vila Atlantica and

from 0 to 16 per cent. at Mogí das Cruzes. In Rio Grande do Sul, this species is relatively more common on the coast (2 to 30 per cent.) than in the interior, where it is quite rare. Furthermore, the frequencies of this species are subject to violent fluctuations at different seasons.

5. *DROSOPHILA NEBULOSA*

Drosophila nebulosa Sturtevant is a member of the *willistoni* species group of the subgenus *Sophophora*. Its distribution region extends from Texas in the North (Patterson and Wagner, 1943) to at least Buenos Aires in the South (Pavan, unpublished). No Drosophila collecting has ever been made south of Buenos Aires. In Brazil, *D. nebulosa* is one of the commonest species, particularly in drier savanna and desert regions, less common in the rain forests. Furthermore, the frequencies of *D. nebulosa* relative to those of other species of Drosophila tend to be higher during the warmer and drier than during the wetter and cooler seasons (Pavan, unpublished).

The metaphase plates of *D. nebulosa* show two pairs of metacentric and one pair of acrocentric chromosomes. Pavan (1946) examined the salivary gland chromosomes of a series of strains, and described 9 inversions, all but one of which were found in a single chromosome, the third, which is the acrocentric autosome. In our material, we have observed 7 out of the 8 inversions described by Pavan in the third chromosome, and found 3 new inversions, namely *Ii*, *Jj*, and *Kk*, also in the third chromosome. The single inversion found by Pavan in the X-chromosome was not found in our material.

The frequencies of the inversion heterozygosis in the populations examined by ourselves are given in table 5. Only the gene arrangements *HH*, *Hh* and *hh* were identified both when homozygous and when heterozygous. The inversions *Aa*, *Bb*, *Cc*, *Gg* and *Hh* occur frequently in all the regions studied ; the inversions *Ee*, *Ff*, *Ii*, *Jj* and *Kk* are less common or restricted to only small fractions of the species distribution area.

It may be noted that a group of populations comprising Mucajai, Monjolinho, Pirassununga, Iguassú and Tucuman show high frequencies of the gene arrangement *h*, while Rio Negro, Imperatriz, Salitre and Catuní are characterised by high frequencies of the alternative gene arrangement, *H*. This distribution of the frequencies of *h* and *H* shows no clear correlations with the ecological characteristics of the localities in which the respective samples were collected. Thus, Mucajaí is more similar to Monjolinho, some 2500 km. away, than it is to Rio Negro, less than 500 km. away. Mucajaí, Iguassú and Rio Negro are covered with rain forest, while the other localities have savanna or desert vegetation.

The mean frequencies of the numbers of heterozygous inversions per individual show, on the contrary, a fairly clear correlation with the ecological characteristics of the regions in which the population

TABLE 5

Frequencies (in per cent.) of various inversions found in heterozygous condition, mean number of heterozygous inversions per individual and relative frequencies (in per cent.) of D. nebulosa *individuals in several populations*

Third chromosome inversions	Mucajaí	Rio Negro	Imperatriz Carolina	Salitre 1949	Salitre 1951	Catuní	Monjolinho	Piras-sununga	Iguassú	Tucuman
Aa	38·5	40·0	32·4	40·0	33·0	20·0	32·3	8·7	8·0	6·0
Bb	43·6	54·3	8·1	4·0	3·0	12·7	26·1	8·7	30·0	36·0
Cc	48·7	42·8	32·4	32·0	21·0	7·3	58·5	34·8	27·0	...
Ee	...	...	...	...	...	...	4·6	...	1·0	...
Ff	...	...	5·4	...	...	...	...	4·3	4·0	...
Gg	41·0	42·8	2·7	11·0	3·0	...	26·1	26·1	24·0	23·0
HH	12·8	48·6	64·9	66·0	56·0	72·7	16·9	17·4	13·0	1·0
Hh	38·5	37·1	29·7	32·0	35·0	21·8	46·1	34·8	31·0	24·0
hh	46·1	14·3	5·9	2·0	9·0	6·5	36·9	47·8	56·0	75·0
Ii	23·0	...	...	...	...	...	...	...	...	...
Jj	...	...	16·2	...	...	...	...	...	...	...
Kk	2·5	14·3	...	...	...	...	...	...	...	...
Mean per individual	*2·36±0·19*	*2·29±0·15*	*1·30±0·16*	*1·20±0·11*	*0·90±0·10*	*0·62±0·09*	*1·94±0·12*	*1·17*	*1·25±0·08*	*0·86±0·08*
Flies tested	39	35	37	100	100	55	65	23	100	100
Species frequencies	1·4-71·5	1·8	51-63	52·4	54·3	26·7	5·2-63·8	0·1-29	8·8	10·3

samples were taken. The highest frequencies are observed at Mucajai, Rio Negro, Monjolinho, and Imperatriz. The first and the second of these localities are covered with exuberant rain forests, but are close to margins of savannas. On Rio Negro in particular, *D. nebulosa* proved to be commoner in drier localities than in wetter ones (up to 50 per cent. of the total catch of Drosophila flies on the beach of Tatuaquara, near Manaus). Monjolinho and Imperatriz lie in ecologically highly complex regions, on or near the ecotones between the savanna and the rain forest, or gallery forest, vegetations. By contrast, Salitre and Catuní, which show low frequencies of inversions, are in a desert (caatinga) region, which is dry and nearly lifeless during more than six months per year. Although *D. nebulosa* is the commonest species of Drosophila in this desert, it certainly can occupy there only a very limited number of ecological niches. It should be noted that *D. willistoni* has also shown the lowest numbers of heterozygous inversions per individual in this region (da Cunha, Burla and Dobzhansky, 1950). Finally, Tucuman lies at the foot of the Andes, presumably near the margin of the distribution area of the species. The population sample was collected there in an isolated forested region having a limited variety of plant species.

6. *DROSOPHILA BANDEIRANTORUM* AND *DROSOPHILA PALLIDIPENNIS*

Drosophila bandeirantorum Dobzhansky and Pavan and *D. pallidipennis* Duda are species with no clear affinities among the known species of Drosophila. The former has a rather restricted known geographic distribution area in southern Brazil. The latter has been recorded from the State of São Paulo, in southern Brazil, as well as from Mexico, but has so far not been encountered in the Amazon Basin. Both species are fairly rare, and appear to be specialised to occupy a restricted variety of ecological niches (Dobzhansky and Pavan, 1950). Their salivary gland chromosomes are among the best found in any species of Drosophila, and this served as a stimulus to study them for inversions.

In 441 examined individuals of *D. bandeirantorum*, two kinds of inversions were found, one in each, the second and the third chromosomes (autosomes). In *D. pallidipennis*, Patterson and Dobzhansky (1945), Freire-Maia and Engel (1949), and ourselves found two inversions, both of them in the same autosome (overlapping inversions). The mean frequency of heterozygous inversions per individual of *D. bandeirantorum* proved to be $0{\cdot}49 \pm 0{\cdot}02$. The material examined came from 4 populations in the State of São Paulo. In the 70 individuals of *D. pallidipennis*, from Pirassununga, in the State of São Paulo, examined by Freire-Maia and Engel (1949) and ourselves, the mean number of heterozygous inversions per individual proved to be even smaller, namely $0{\cdot}07 \pm 0{\cdot}04$.

7. DISCUSSION

The data presented above are in a general agreement with the hypothesis that the amount of genetic variability present in a population is positively correlated with the number of ecological niches which this population exploits. Thus, the common and widespread species, *D. polymorpha*, has more heterozygous inversions per individual than its closest but less common relative, *D. cardinoides*. Similarly, the common *D. guaramunu* has more inversions than its less frequent relative, *D. griseolineata*. In *D. nebulosa* the populations which inhabit the ecologically limited and highly exacting desert environments show fewer inversions than do the inhabitants of richer and more diversified rain forest and savanna environments (*cf.* da Cunha, Burla and Dobzhansky, 1950).

Several reservations must, however, be made. In the first place, only closely related species, or races within a species, can profitably be compared. In different groups of organisms the genetic variability takes different forms, and chromosomal inversions are characteristic of the adaptive polymorphism in some sub-divisions of the animal and plant kingdoms but not in others. Even within the genus Drosophila, some species groups seem to be richer in inversions than others (thus, the subgenus *Sophophora* seems to have more inversions than the subgenus *Drosophila*). Some species have inversions in all chromosomes (e.g. *D. willistoni* and relatives, *D. robusta*, *D. virilis*), while other species have them concentrated in a single or in few chromosomes (e.g. *D. pseudoobscura*, *D. persimilis*, *D. nebulosa*).

Furthermore, given the present imperfect understanding of the ecology of Drosophila, our ability to evaluate the relative numbers of the adaptive niches controlled by a population is limited. By and large, an abundant and widespread species is likely to be ecologically more versatile than a rare one restricted to only few habitats. It stands, however, to reason that an ecologically specialised species may occur more commonly than an ecologically versatile one, provided that the special environment of the former is available in many places within a certain geographic region. Situations apparently contradictory to the hypothesis are, then, not unexpected, but, provided that the hypothesis is correct, they should be less frequent than situations conforming to the hypothesis. Such an apparent contradiction may be seen in table 1. *D. polymorpha* has significantly more inversions per individual at Vila Atlantica than at Pirassununga or at Mogí das Cruzes. And yet this species reaches at times much higher frequencies at the latter than at the former localities. It may be significant in this connection, that the population size undergoes violent fluctuations at Pirassununga and at Mogí das Cruzes, reaching up to 22 per cent. of the total Drosophila catch during the summer, but becoming very rare during winter. At Vila Atlantica the population of *D. polymorpha* does not undergo such sharp expansions and contractions at different seasons.

8. SUMMARY

Natural populations of several species of Drosophila were examined for the incidence of inversion heterozygotes among the individuals composing them. Inversion heterozygotes proved to be relatively more frequent in *Drosophila polymorpha* than in its close relative, *Drosophila cardinoides*, and more frequent in *Drosophila guaramunu* than in the neighbouring species, *Drosophila griseolineata*. *Drosophila polymorpha* and *Drosophila guaramunu* are more common and widespread in southern Brazil than are *Drosophila cardinoides* and *Drosophila griseolineata*. In *Drosophila nebulosa*, populations which inhabit ecologically rich and diversified environments of savannas and rain forests have more inversions than do geographically or ecologically marginal populations. These facts stand in accord with the hypothesis that the amount of adaptive polymorphism present in a population is positively correlated with the variety of environments which this population controls.

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CHROMOSOME BEHAVIOUR IN WHEAT MONOSOMICS

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1. INTRODUCTION

THE use of monosomics, $6x-1$ plants, for genetical studies and for the transference of desirable characteristics from related genera into the hexaploid wheats (*Triticum vulgare*) has become an important part of the cereal breeding programme. Some particular cytological studies of the monosomes (the deficient chromosomes) have been made : Sears (1939, 1944, 1952, 1952*a*), Unrau (1950), Unrau *et al.* (1950) and Morrison and Unrau (1952). As suggested in an earlier report there are several major problems requiring clarification.

The B-series of speltoid wheats as classified by Nilsson-Ehle in 1921 has been associated with the loss of one chromosome (Winge, 1924). A review of work on this strain of wheat has been given by Huskins and Sander (1949). In the Sear's monosomic series the speltoid chromosome is number nine (mono-IX). Much of the behaviour of the other monosomes has been inferred from the reported behaviour of this monosome. The project reported in this paper was undertaken with the view of comparing the meiotic behaviour of all the monosomes.

One phase that is not yet clear is the behaviour of the chromosomes in the pollen grains (P.G's.). From time to time inferences have been made that P.G's. with varying numbers of chromosomes are produced or that telocentrics or isochromosomes may be transmitted through the male gamete, but no serious attempt has been made to study this phase of development. By the use of a new technique it was possible to make an extensive record of the behaviour of the chromosomes in the P.G's.

The morphology of the various monosomes was also compared. This was done to ascertain if any homologies were present and also as an aid to identification of telocentric or fragmented chromosomes in the P.G's. or in the somatic complement of root-tips.

2. METHODS AND MATERIAL

All investigations of P.M.C's. were made from acetocarmine squashes after whole spikes were fixed in Carnoy. Root-tips were pretreated with monobromonaphthalene, fixed in 2 BD, and then prepared as Feulgen squashes. Pollen grain preparations were made using the technique outlined previously (Morrison, 1953). For studies of megasporogenesis, ovules that had been fixed in Carnoy were embedded in paraffin and stained with crystal violet. Sections were cut at 12 microns.

The 21 monosomic lines of wheat are those developed and distributed by Sears, *i.e.* monosomics of *T. vulgare* var. Chinese Spring. The chromosomes are numbered from I to XXI and the deficient monosomes abbreviated to mono-I, mono-II, etc. Mono-XIV was not available for all studies.

3. CHROMOSOME MORPHOLOGY IN *T. VULGARE*

With the exception of the satellite chromosomes I and X it was not possible to identify each of the 21 different chromosomes by

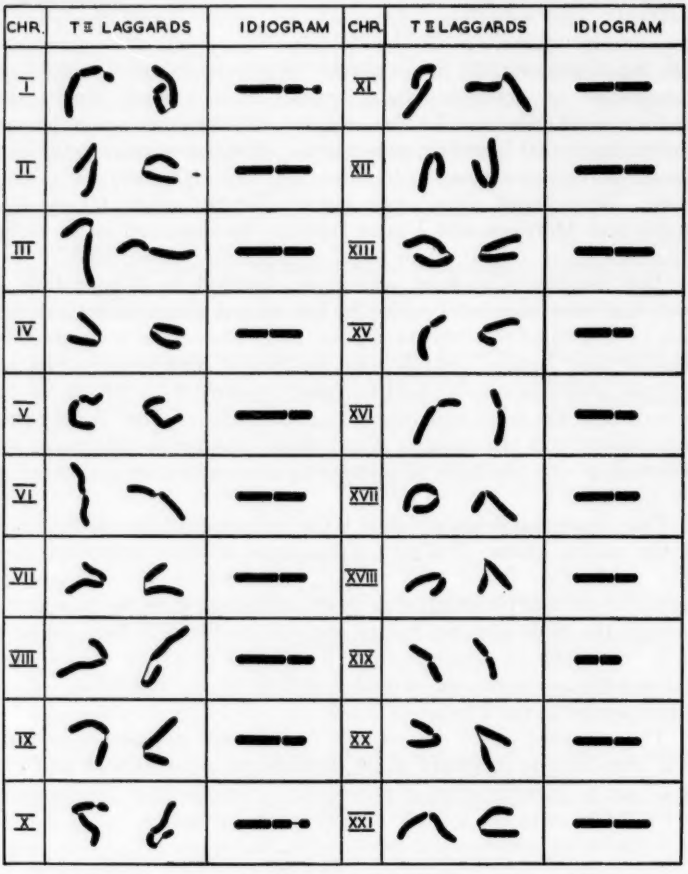

FIG. 1.—The morphology of the chromosomes of Chinese Spring; XIV is omitted. Drawings were made from univalent laggards at TII. Magnification × 1300.

morphology studies of the root-tip metaphases of the monosomics. A comparison of the chromosomes was made however from the lagging univalents at telophase II (TII). The sizes and shapes (fig. 1) agree in general with the haploid set observed in the P.G's. and with the

root-tip complement. It has long been known that IX has unequal arms. According to Sears and Camara (1952) VII is also unequal. Mono-I is sometimes visible at MI as a satellited univalent.

The methods used in the past for studying the morphology of the chromosomes have been laborious and not always fruitful. Differences in sizes are not outstanding and divergence in arm length occurs to the same degree in too many chromosomes. As well, all reports are conditioned by cytological procedures. In the earlier works (Kagawa, 1929; Bhatia, 1938; and Levitsky *et al.*, 1939) two or three constrictions per chromosome were noted. Three pairs of satellite chromosomes have been recognised by Bhatia (1938), Pathak (1939) and Camara (1944) and two pairs by Levitsky *et al.* (1939) and Tjio and Levan (1950).

At diplotene-diakinesis I have observed from one to three bivalents attached to the nucleolus. This indicates that some organising regions are weaker than others and only operate occasionally. In like manner only rarely are six nucleoli seen in a resting nucleus. The constriction in chromosome X is never visible at MI and is not constant at AII. This variability in appearance and function of the nucleolar arm may thus be an expression of the evolutionary change in this polyploid (*cf.* Darlington, 1937, p. 228 and Woods, 1937). If Larson (1952) is correct and I and X are both from one genome then the satellite chromosomes from the other genomes must be considerably changed in appearance.

From the compensating effects of tetrasomics, Sears (1952*b*) has suggested several homœologous series. The limitation of attempting a comparison by shape of chromosomes is immediately apparent in the series I, XIV, XVII. Homœologies are uncertain because of evolutionary changes.

4. MEIOSIS IN POLLEN MOTHER CELLS

Metaphase pairing.—At MI the 41 chromosomes usually form 20 bivalents and one univalent (table 1). Contrary to Sánchez-Monge and Mac Key (1948) no higher associations were found. There is no evidence for any serious degree of variation among the monosomes.

The asynaptic condition observed in four cells of mono-IX is the result of either an environmental stimulus or a mutation in the sporogenous tissue. It is not comparable to the desynapsis in nulli-III plants or to the asynapsis reported by Huskins and Hearne (1933) or Li *et al.* (1945) where all the cells within an anther showed a varying amount of failure in pairing.

Univalents at AI and TI.—At AI, the univalents may lag or they may not; they may divide or misdivide, and at TI they may be included or excluded. Frequently after division both chromatids are swept to one pole. Division of the monosome occurs in only slightly more than half of the cells. Misdivision of the centromeres

resulting in telocentrics or isochromosomes takes place in 12 per cent. of the cells (table 2). With the exception of mono-XII which is slightly low, there are no great differences among the monosomes.

TABLE 1

Chromosome pairing at metaphase I in eight monosomic lines

Monosomic	MI pairing			No. of cells examined
	$20^{II}\,1^{I}$	$19^{II}\,3^{I}$	$18^{II}\,5^{I}$	
I	194	4	...	198
II	72	5	...	77
IV	385	6	...	391
VIII	88	5	3	96
IX *	161	4	...	165
XV	129	2	...	131
XVI	210	4	1	215
XIX	168	1	...	169
Total	1407	31	4	1442
Per cent.	*97·6*	*2·1*	*0·3*	...

* In another sample from this plant in which most of the cells were at interphase, there were three delayed cells at MI where pairing had completely failed. In one other cell pairing had been reduced to two bivalents and 37 univalents.

TABLE 2

Frequencies of misdivision and formation of micronuclei at interphase in eleven monosomic lines

Monosomic	Misdivision of univalents				Micronuclei in dyads	
	TI		TII			
	No. of cells examined	Per cent. with misdivision	No. of cells examined	Per cent. with misdivision	No. of cells examined	Per cent. with micronuclei
I	64	*9·4*	60	*33·3*	357	*38·9*
III	...	...	52	*27·0*	286	*40·6*
V	87	*14·9*	23	*17·4*	327	*43·1*
VIII	152	*13·8*	46	*13·0*	325	*31·7*
IX	68	*13·2*	73	*21·9*	293	*34·1*
X	120	*10·0*	28	*25·0*	534	*33·9*
XI	255	*13·3*	135	*11·1*	640	*43·9*
XII	103	*5·8*	...	...	475	*42·3*
XIII	112	*11·6*	106	*14·2*	438	*49·1*
XX	...	...	...	...	225	*53·7*
XXI	53	*17·0*	70	*11·4*	...	...
Total	1014	12·1	593	17·7	3900	41·0

The frequencies of misdivision previously reported for mono-IX are 1·7 per cent. (Sánchez-Monge and Mac Key, 1948) and 39·7 per

cent. (Sears, 1952). It is evident that different methods of scoring have been used. These workers as well reported that the univalent divided in nearly all the cells (96 and 97 per cent.) which is not in

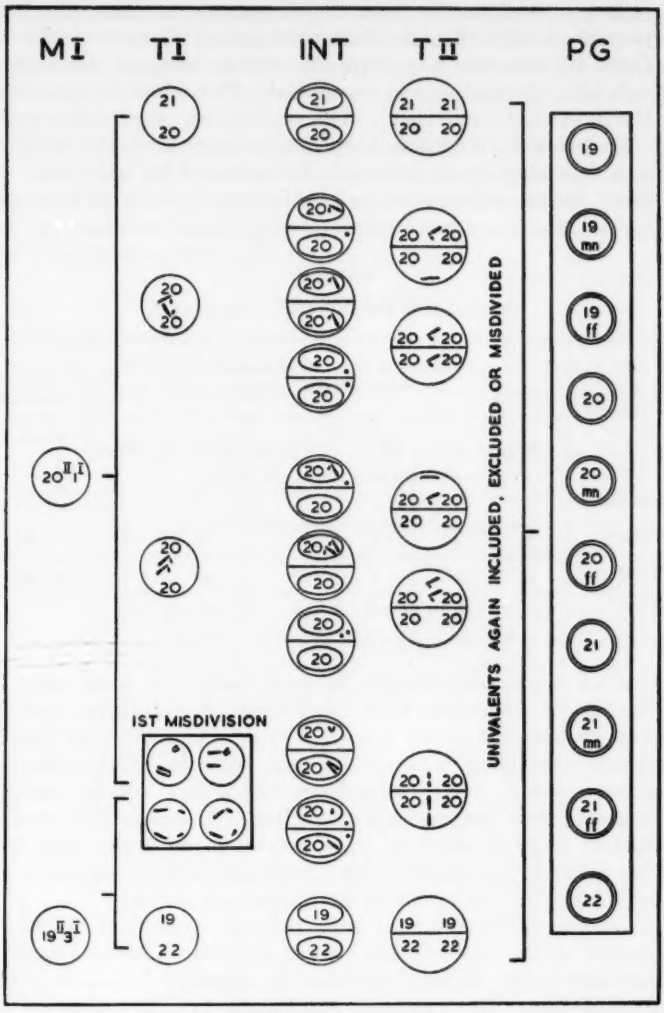

FIG. 2.—The chief variations in behaviour of the univalent chromosomes at meiosis and the resulting chromosome numbers in the P.G's. Each phase is described in the text. Micronucleus (mn) formation in the tetrads has been described previously (Morrison and Unrau, 1952).

agreement with my results. In any event misdivision frequencies cannot be estimated too accurately because of questionable phases and observations at critical times of division.

Interphase.—Univalent laggards or fragments from misdivision not included in the TI nuclei form micronuclei (table 2). One or two and less frequently three or four micronuclei per dyad were observed. There is no correlation between the frequencies of misdivision and the proportion of dyads with micronuclei among the monosomes.

There is, however, a positive correlation between the number of dyads with micronuclei and the number of tetrads with micronuclei (*cf.* Morrison and Unrau, 1952). This confirms my original assumption that the inclusion or exclusion of chromosomes from the telophase nuclei is dependent upon the specific behaviour of the univalent.

Second division.—Chromosomes at AII were counted in some cells (table 3). Actual counts were frequently limited to one sister half

TABLE 3

Distribution of the chromosomes at anaphase II

Monosomic	Anaphase II nuclei with various chromosome numbers						Total anaphase groups counted
	19	20	20+*f*	20+2*ff*	21	22	
Mono-III .	...	49	1	1	7	...	58
All others .	2	49	3	...	29	1	84
Total .	**2**	**98**	**4**	**1**	**36**	**1**	**142**
Per cent. .	*1·4*	*69·0*	*2·8*	*0·7*	*25·4*	*0·7*	...

because of non-synchronisation in both cells. In some cells the chromatids or fragments from misdivision of univalents were incorporated into the polar groups. More frequently the lagging univalents were lying between the two poles in which case they were not counted. It is impossible to tell if they will be included or excluded from the microspore nucleus. Although AII is not a satisfactory stage at which to make a great many counts or a comparison between monosomes it does given an accurate picture of the distribution of the chromosomes into microspore nuclei.

Univalent chromatids that arise through equational division at AI cannot divide again in the second division. Like the products of the delayed first division they may be included, excluded or they may misdivide. The increased misdivision at TII (table 2) will result in a general increase in telocentrics. Isochromosomes formed at first division may be separated into telocentrics. However, reunion of broken ends in the resting microspores may restore both isochromosomes and monosomes if both telocentrics are included within one nucleus.

At MII some of the micronuclei formed at the first division become

active. They behave as lagging chromatids. Other micronuclei which are isolated near the periphery of the cell are delayed in development. They have the appearance of prophase chromosomes when the others are undergoing division. They are not included in the telophase nuclei but again form micronuclei. Most of the micronuclei have disappeared from the P.G's. by the time the cytoplasm becomes vacuolated. Presumably the micronuclei are absorbed by the cytoplasm.

Whole chromosomes or fragments that are delayed in division are sometimes isolated from the tetrads as microcytes (*cf.* Frankel, 1949). These microcytes develop into small pollen grains. Although only one-eighth as large, they are similar in appearance to the normal P.G's. but have no divisions.

5. POLLEN GRAINS

Metaphase.—Although varying chromosome numbers in the P.G's. have been postulated to account for progeny results this is the first report of actual observations (table 4). The number in any P.G.

TABLE 4

Distribution of the chromosome numbers at 1st division in the P.G's. for twenty monosomic lines

Monosomic	P.G's. with various chromosome numbers											No. of P.G's. examined
	18	19		20				21		22	23	
		0*f*	1*f*	0*f*	1*f*	2*ff*	3*ff*	0*f*	1*f*			
I	...	...	...	3	1	1	...	20	...	...	...	25
II	...	...	...	31	1	1	...	16	1	...	...	50
III	...	...	...	40	2	...	...	9	...	...	...	51
IV *	...	1	1	92	5	...	...	29	...	...	...	128
V	...	1	...	8	1	...	...	18	...	...	...	28
VI	...	...	...	16	1	...	...	12	...	1	...	30
VII	1	...	...	3	...	...	...	23	1	1	...	29
VIII *	1	1	...	102	8	1	...	42	...	1	...	156
IX *	1	1	1	78	7	1	...	51	1	...	...	141
X *	...	...	...	39	4	3	...	35	...	1	1	83
XI	...	...	...	30	...	1	...	9	1	...	...	41
XII	...	...	...	34	...	...	...	5	...	...	1	40
XIII	...	...	...	35	1	1	...	34	...	...	1	72
XV *	...	1	...	48	10	...	...	16	...	...	...	75
XVI	...	1	...	21	...	...	...	3	...	...	...	25
XVII *	...	...	...	72	5	...	...	30	...	...	...	107
XVIII	...	...	...	18	2	...	...	10	...	1	...	31
XIX	1	1	...	52	6	...	...	15	...	1	...	76
XX	...	...	...	66	10	2	...	15	...	...	...	93
XXI	...	1	...	24	1	...	1	1	...	...	...	28
Total	4	8	2	812	65	11	1	393	4	6	3	1309
Per cent. of Total	0·3	0·6	0·1	62·0	5·0	0·8	0·1	30·0	0·3	0·5	0·2	

Mean number of chromosomes per P.G. = 20·3.

* Combined results for two years.

depends upon the inclusion or exclusion of lagging univalents in that particular microspore nucleus.

The 1951 results showed significant differences among the monosomes for the formation of P.G's. with 20 or 21 chromosomes. From more extensive studies in 1952 it is evident that results for the two years vary considerably (table 5) and that no specific ratio can be established from the results of the small samples.

TABLE 5

Distribution of the chromosome numbers at 1st division in the P.G's. for two years, for mono-IX and mono-X

Mono. year	P.G's. with various chromosome numbers										No. of P.G's.	No. of chrs.*	Mean per P.G.
	18	19		20			21		22	23			
		0*f*	1*f*	0*f*	1*f*	2*ff*	0*f*	1*f*					
IX-1951	...	...	...	17	2	...	22	...	...	...	41	842	20·5
IX-1952	1	1	1	61	5	1	29	1	...	...	100	2026	20·3
X-1951	...	...	...	19	3	2	7	...	1	1	33	672	20·4
X-1952	...	...	...	20	1	1	28	...	...	...	50	1028	20·6

* Omitting fragments.

Some of the apparent heterogeneity among the different monosomes may be due to differences in the stage of anther development. If the P.G's. with 20 chromosomes are sufficiently unbalanced they will be delayed in coming to mitosis. Then if the anthers are examined late in the cycle a bias in the favour of the 20-chromosome P.G's. will be introduced. No estimate of this bias could be made by counting the number of uni-nucleate and bi-nucleate P.G's. present.

Disregarding fragments and using the total for all monosomes (table 6) the ratio of the various numbers agrees with results for AII. As expected, the frequency of 18, 19, 22 and 23 is low, but these P.G's. are produced in sufficient numbers to warrant attention as possible gametic sources of variation.

Because of the loss of chromosomes as micronuclei, more P.G's. with 20 than with 21 chromosomes are expected. Even in the tetrads with no micronuclei two of the microspores will be deficient. As 50 per cent. of the tetrads have one or two micronuclei (Morrison and Unrau, 1952) the ratio of P.G's. with 21 to 20 chromosomes should be approximately 25 : 75. My results are not greatly divergent from this ratio.

Deficient chromosomes were tabulated as fragments because it was not always possible to discriminate between telocentrics and

chromosomes with ruptures near the centromere. Of all fragments, 60 per cent. were certain telocentrics, another 15 per cent. were inferred and 25 per cent. were doubtful. There is no correlation between misdivision frequencies and number of fragments observed for the various monosomes.

TABLE 6

Distribution of the chromosome numbers and a comparison of frequency of fragments at 1st division in the P.G's.

ff	P.G's. with various chromosome numbers							No. of *ff*
	18	19	20	21	22	23	No. of P.G's.	
0	4	8	812	393	6	3	1226	0
1	...	2	65	4	...	...	71	71
2	...	...	11	...	...	...	11	22
3	...	...	1	...	...	...	1	3
Total	4	10	889	397	6	3	1309	96
No. of cells with *ff*	...	2	77	4	...	...	83	...
ff per cent.	...	*20·0*	*8·7*	*1·0*	...	...	*6·3*	...

TABLE 7

Anaphase and telophase of 1st division in P.G's. in six monosomic lines

Monosomic	Anaphase or telophase in the P.G's.		
	Normal with no laggards	With laggards	Total examined
I	68	2	70
VII	7	1	8
VIII	65	1	66
XV	18	0	18
XX	34	2	36
XXI	65	0	65
Total	257	6	263
	97·7 per cent.	*2·3* per cent.	...

No actual isochromosomes were recorded although two probables were seen. It would be difficult to distinguish them from chromosomes with median centromeres except those duplicated for the trabant arm of I or X. In three P.G's. chromosomes deficient for only a portion of an arm were seen.

An examination of the second mitotic division showed that P.G's. with 20 and 21 chromosomes were undergoing division. Results were not tabulated because the numbers of observations were limited. The smallness of the chromosomes also prevented an accurate analysis.

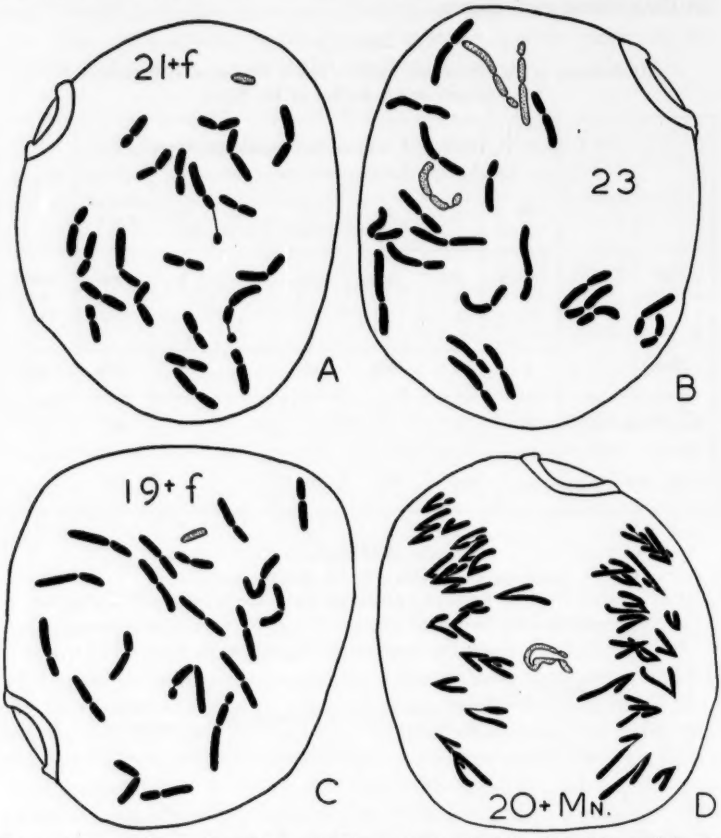

FIG. 3.—Four pollen grain mitoses, odd chromosomes stippled. The P.G. wall is not drawn to scale. Magnification × 1300.

Anaphase and Telophase.—Studies were made of anaphase and telophase in six monosomic lines (table 7). No attempt has been made to compare the various monosomes statistically because of the small numbers for some monosomes. The frequency of abnormal behaviour calculated from the total is probably near correct for all monosomes.

The six laggards can be classified into two types :—

(1) Cells with extra nuclear material out of phase. Micronuclei carried over into the P.G's. become active but are delayed

in development (fig. 3D). The action of the micronucleus is comparable to the action it frequently undergoes at interphase. The two laggards in mono-XX were of this kind.

(2) Cells with centric fragments or chromosomes that have sub-effective centromeres (some may be acentric fragments) which were included in the microspore nucleus at TII. The other four were of this kind (fig. 4J).

Darlington (1940) proposed non-disjunction of telocentrics at P.G. mitosis to account for the production of isochromosomes in *Fritillaria*. Sánchez-Monge and Mac Key (1948) have proposed a similar mechanism in wheat. No support for this theory was found. Nor is there any support for the second postulate of Rhoades (1940) whereby isochromosomes produced at meiosis become micronuclei which are carried over to the P.G. and then become active. As Sears (1952*a*) has suggested, isochromosomes and telocentrics arise directly through misdivision at meiosis.

6. MEIOSIS IN EMBRYO SAC MOTHER CELLS

Observations of meiosis were made from sectioned ovules of mono-VII, XVI, XX and XXI. The behaviour of the univalent (fig. 4D) is similar to its behaviour in the P.M.C's., *i.e.* it lags, divides and forms micronuclei, etc. The plane of division in one dyad is at right angles to the other however, and this results in a T-shaped tetrad of megaspores (fig. 4E).

The wheat gametophyte is monosporic and it is always the innermost cell (cell number 4) that develops into the embryo sac. Micronuclei are found in all four megaspores. Out of a total of 48 tetrads 79·2 per cent. had one or more micronuclei. In mono-XX out of 27 tetrads 81·5 per cent. had micronuclei.

If no micronuclei are present we cannot be certain whether the terminal megaspore or the first two have 21 chromosomes. Again, if there is one micronucleus in the third megaspore it does not indicate the deficiency or completeness of cell number four. Probably the best check on the formation of deficient or normal gametes can be made from crossing and selfing experiments. Sears (1944) has shown that in general 75 per cent. of the female gametes are deficient.

7. PROGENY

A record was kept of the chromosome number of all plants grown to maturity (table 8). Results for individual monosomics are not of sufficient magnitude to warrant a comparison. Of the total, the ratio of normal to monosomic plants is slightly higher than expected but the number of nullisomics compares favourably with previous reports (Sears, 1944 ; and Smith *et al.*, 1949).

As the amount of zygotic elimination and embryo abortion is practically nil we must assume excessive certation, partial pollen sterility at later stages or slower pollen tube growth of defective P.G's., to account for the ratio. With a ratio in the female of monosomic to normal as 75 : 25, then P.G's. with 20 chromosomes can be only one-thirtieth as efficient as the P.G's. with 21 chromosomes or there would be many more nullisomics produced.

TABLE 8

Progeny grown from monosomic seed

	Type of plant and chromosome number				Total
	Disomic 42 chromosomes	Monosomic 41 chromosomes	Nullisomic 40 chromosomes	Other Aberrants *	
No. of plants .	68	101	2	6	177
Per cent. .	*38·4*	*57·1*	*1·1*	*3·4*	...

* These plants were as follows :—

i. 1 with 40 plus an isochromosome.
ii. 2 with 41 plus an isochromosome.
iii. 2 with 41 plus a telocentric.
iv. 1 with 40 plus a telocentric plus a chromosome with an interchange that arose through fusion of telocentrics, a process to be described later.

With 6·3 per cent. of all P.G's. having fragments it is not surprising that a proportion of all progeny from selfed monosomics have either telocentrics or isochromosomes. Deficiencies can be transmitted through the egg as well. Of chief importance to the plant breeder is the phenotypic expression of small or large deficiencies or duplications which can arise through the processes of misdivision of the univalent chromosome at meiosis.

8. SUMMARY

1. The 21 expected monosomics in *T. vulgare* can be identified by crossing. Only two or three of them can be distinguished by chromosome structure.

2. In all monosomics 20 bivalents are formed in 97 per cent. of the P.M.C's. Chromosome mosaics, both hypoploid and hyperploid cells, were found in monosomic and disomic lines (see Appendix).

3. The univalents either divide or misdivide and either lag or do not lag at either first or second telophase.

4. Isochromosomes in wheat arise directly through misdivision at meiosis and not indirectly through non-disjunction in the P.G's.

5. P.G's. with 18-23 chromosomes are formed and undergo mitosis.

Six per cent. of the P.G's. contain fragments, most of which are telocentrics.

6. Over twice as many P.G's. with 20 as with 21 chromosomes are produced but the former are only 1/30th as effective as the latter in fertilisation.

APPENDIX

Chromosome mosaics at metaphase I.—At MI 32 cells with a reduced or aneuploid number and two cells with a hyperploid number were observed (table 9). Following the terminology of Sachs (1952) who found aneuploid cells in *Triticum* hybrids, these abnormalities are classed as chromosome mosaics.

Frequencies are not intended to show differences among the monosomes because in other slides from the same spikes no mosaics were found. Normally there are over 500 P.M.C's. in a squash of three anthers but because of non-synchronisation only about 300 at full MI are expected. In those slides where the number of cells at MI is small the predominating stage was telophase or interphase. Presumably the unbalanced cells are less efficient and thus are delayed.

TABLE 9

Distribution of chromosome mosaics in pollen mother cells

Plant type	Total no. of cells at MI in squash of 3 anthers	Chromosome mosaics		
		No. of aberrant cells	Chromosome no. in the aberrant cells	Pairing arrangement
DISOMICS				
A	36	1	29	$8^{II}\ 13^{I}$
B	43	2	29	$9^{II}\ 11^{I}$
MONOSOMICS				
I	198	1	77 †	$37^{II}\ 3^{I}$
II	83	6	30	$10^{II}\ 10^{I}$
VI	222	3	32	$12^{II}\ 8^{I}$
VIII	96	1	23	$5^{II}\ 13^{I}$
		1	16	$2^{II}\ 12^{I}$
XV	200	2	19	$6^{II}\ 7^{I}$
XVII	73	14	32	$12^{II}\ 8^{I}$ / $11^{II}\ 10^{I}$
XVIII	1021 *	2	32	$12^{II}\ 8^{I}$
MONOSOMIC				
XIV+ISO—XIV	93	1	73 †	$35^{II}\ 3^{I}$

* Count from 3 florets. † Hyperploid cells.

In mono-XVII evidently the abnormality must have occurred at least four cell generations prior to meiosis. Premeiotic mitoses were examined in two plants but out of 275 cells at anaphase or metaphase no spindle irregularities were observed. There were three doubtful cells, two with a bridge and one with a micronucleus. With abnormal divisions occurring so early in the anther development it is difficult to get cytological proof. It is probable, however, that the aneuploid cells are caused by multipolar spindles as suggested by Sachs (1952). Hyperploid cells can be explained by fusion of two spindles.

Because the chromosome mosaics appeared in disomic plants it may be an indication of the instability of Chinese Spring. It may also suggest that the chromosome mosaics may be the result of either environmental stress or gene mutation and that close scrutiny would reveal their presence in other non-hybrid material. With the rare occurrence of chromosome mosaics and with the competition they would suffer it is unlikely that many aneuploid cells would form viable P.G's. However, it is a possibility. They thus may prove a source of variation for plant breeders. In any event they offer an explanation for some of the abnormal chromosome numbers reported from time to time in cereals.

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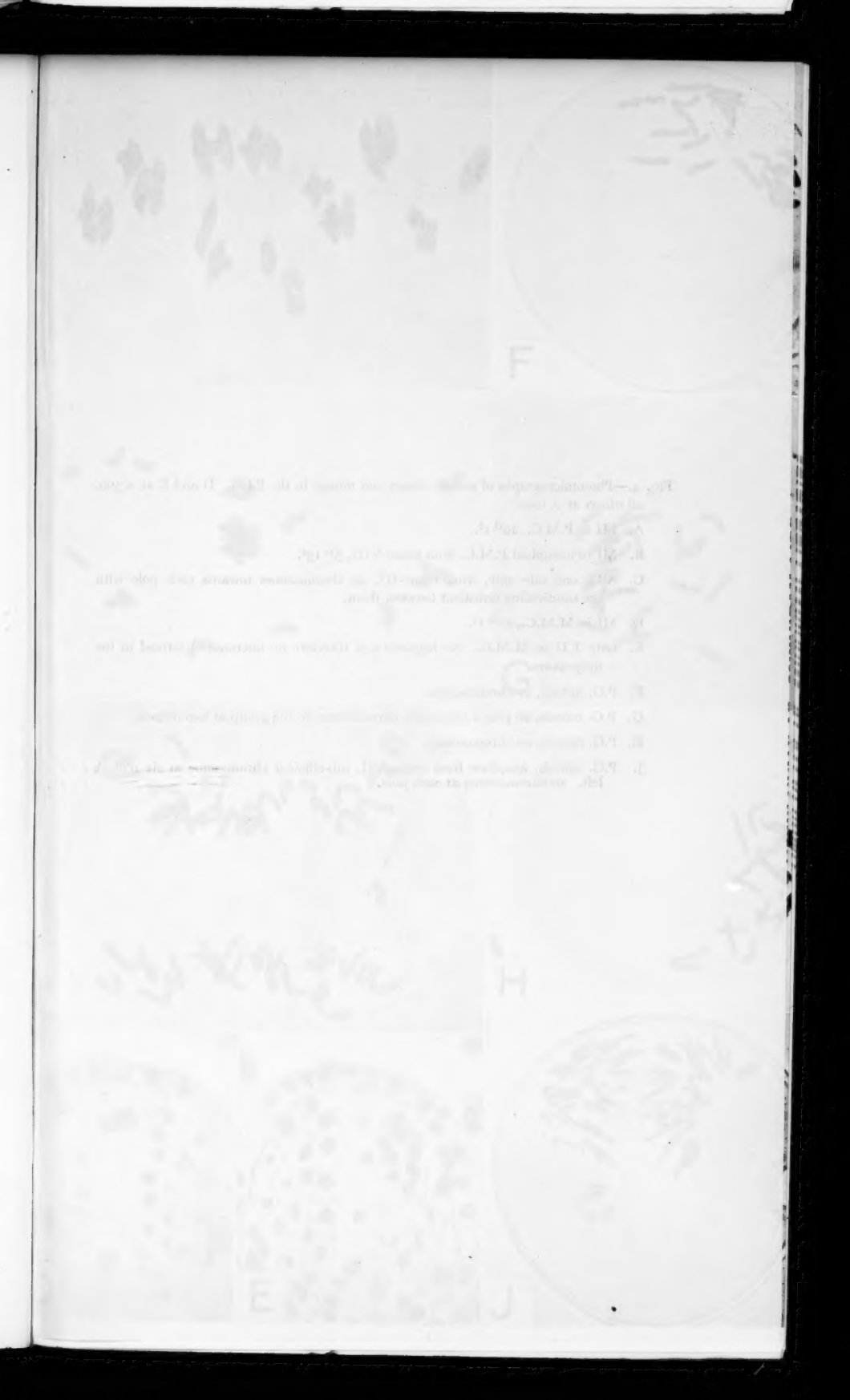

FIG. 4.—Photomicrographs of meiotic stages and mitosis in the P.G's. D and E at ×500, all others at ×1000.

A. MI in P.M.C., $20^{II}\ 1^{I}$.

B. MI in aneuploid P.M.C. from mono-VIII, $5^{II}\ 13^{I}$.

C. A II, one side only, from mono-III, 20 chromosomes towards each pole with the misdividing univalent between them.

D. MI in M.M.C., $20^{II}\ 1^{I}$.

E. Late T II in M.M.C. No laggards and therefore no micronuclei formed in the megaspores.

F. P.G. mitosis, 21 chromosomes.

G. P.G. mitosis, 20 plus a telocentric chromosome, in the group at two o'clock.

H. P.G. mitosis, 20 chromosomes.

J. P.G. mitosis, anaphase from mono-VII, sub-efficient chromosome at six o'clock left. 20 chromosomes at each pole.

A
B
C
D
E
F
G
H
J

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THE CHROMOSOMES OF *CHRYSANTHEMUM*, III: MEIOSIS IN *C. ATRATUM*

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1. INTRODUCTION

GERM CELLS with unreduced nuclei arise in plants and animals from irregularities in meiosis, owing to genotypic causes or to structural hybridity. Many types of upset in the meiotic cycle have been reported and we are now coming to know the kinds of errors by which these variations arise.

Genotypically controlled irregularities are often associated with reduced precocity of the early meiotic stages. A new type of irregularity, intermediate between normal meiotic and completely asynaptic behaviour, has now been found in *Chrysanthemum atratum*.

C. atratum exists in two forms, diploid $2n = 18$ and autotetraploid $2n = 36$. All nine chromosomes of the basic set have nearly median centromeres. Meiosis in the diploid form is normal. The meiotic abnormalities were found in four tetraploid plants, probably of one clone, obtained from the Royal Horticultural Society, Wisley. The similarity in behaviour of these plants suggests that they have arisen from a clone.*

2. MEIOSIS

Normal cells.—Pairing and chiasma formation in normal cells is like that in other autotetraploid species of *Chrysanthemum*. Associations of two, three and four chromosomes are observed with varying numbers of univalents; bivalents each had 1-3 chiasmata (fig. 1). Both first and second meiotic divisions follow the usual course, the univalents dividing at AI. There is the expected variation in chromosome number of the resultant pollen grains.

Precentric cells.—In 63 per cent. of pollen mother cells there were regularly 18 bivalents at MI. These were abnormal in that they showed a precocious splitting of their centromeres as well as a reduced chiasma frequency. In fact each had a single terminal chiasma. Cells behaving in this unusual way have been termed "precentric cells" and the chromosome associations they contain "precentric bivalents." Seven per cent. of cells were intermediate and had both

* A modified Feulgen technique was used for anther squashes with acetic alcohol (1 : 3) as fixative. A hydrolysis time of 4 mins. was sufficient as opposed to the 15 mins. for somatic root-tip squashes (Dowrick, 1952). Anthers were also fixed in a solution of absolute alcohol and saturated ferric acetate in glacial acetic acid (3 : 1) and stained in acetocarmine. Extra iron was unnecessary. Both methods gave satisfactory results.

normal and precentric chromosome associations in the same cell (table, fig. 3).

FIG. 1.—(*a*) First metaphase in a normal pollen mother cell of *C. atratum*. (*b*) First anaphase in a normal cell. × 1200.

Abnormalities are confined to pollen mother cells: root-tip mitoses are normal. The abnormal divisions were present in similar proportions not only in all four plants, but also in all five anthers of

TABLE

Frequency of precentric pollen mother cells at MI

	Cells examined	Normal	Mixed	Precentric
Plant 1 . . .	*91*	26	3	62
Plant 2 . . .	*79*	23	7	49
Plant 3 . . .	*52*	18	4	30
Plant 4 . . .	*107*	31	10	66
Total . .	**329**	**98**	**24**	**207**
Percentage . .	...	30	7	63

the individual florets (table). There is a random distribution of precentric cells. They are scattered throughout the anthers, both in groups and isolated.

The division of the centromeres does not occur until the bivalents are already on the metaphase plate. The arrangement of the bivalents is then normal. Their appearance, with divided centromeres and completely terminalised chiasmata, is quite characteristic (fig. 2 and Plate).

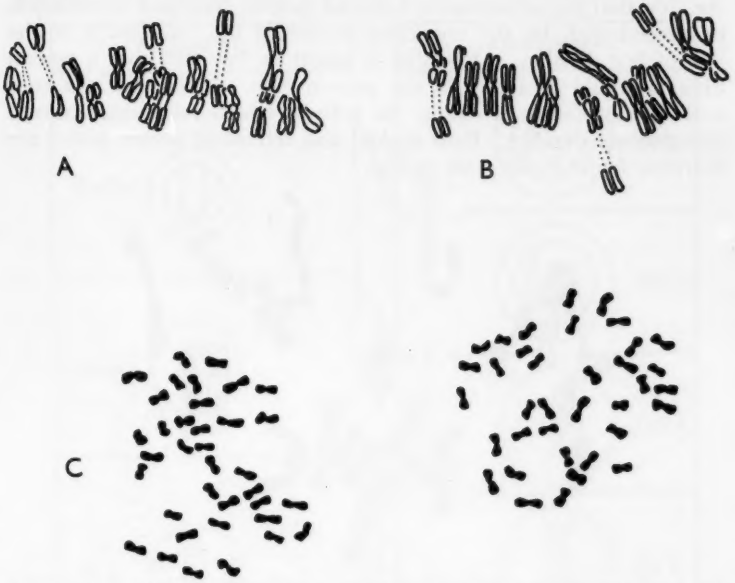

FIG. 2.—(*a*) and (*b*) first metaphase in *precentric* cells. (*c*) First anaphase with precociously split chromosomes resulting from such metaphases. × 1200.

The half-bivalents separate from each other normally, each passing to its respective pole without active repulsion between the divided centromeres of each chromosome. However, having already divided precociously, the centromeres of the chromatids tend, during anaphase movement, to fall apart with the result that at first telophase each nucleus contains 36 chromosomes. The first meiotic division of the abnormal cells does not, therefore, reduce the chromosome number. Nor, since crossing over is near the ends, can it greatly change the genetic constitution of the cell.

In normal cells at the conclusion of first division the chromosomes are, with the exception of the centromere, structurally double. But, as they have single centromeres, they are mechanically single. Consequently, at MII, when the centromeres divide and the two halves of each chromosome separate, the number of centromeres in each daughter cell is half that in the mother cell.

In precentric pollen mother cells, however, where the chromosomes have divided at MI and the half chromosomes have separated at AI,

the interphase chromosome threads are single throughout. They are similar to daughter univalents and, like them, do not divide again during a second meiotic division (Darlington, 1939). Where this applies to all the chromosomes it appears that the second division of the nucleus itself is precluded. This observation lends support to the view that the occurrence of a second meiotic division is determined, or precipitated, by the undivided nature of the centromere at the end of first division. Otherwise it might be expected that a second division would be found in the present case. In fact meiosis ends with the formation of dyads, the cells of which retain the somatic chromosome number. Both diploid and tetraploid pollen grains are therefore found in the same anther.

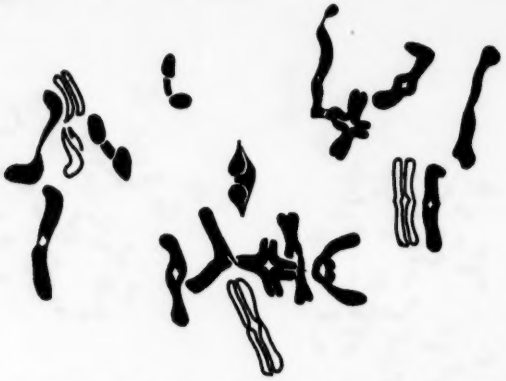

FIG. 3.—First metaphase in cell with both normal and precentric chromosome associations. × 1600.

In the 7 per cent. of cells with mixed chromosome associations their proportions vary (fig. 3). I have been unable to trace their later history but the interphase nuclei arising from them will contain both single and double chromosome threads and, therefore, no doubt, be capable of undergoing a second division. The daughter chromosomes from the precentric bivalents will, presumably, be scattered through the four resultant nuclei.

In 3 of the 207 precentric cells (table), there were bivalents with chiasmata at both ends. Their behaviour is similar to those with a single chiasma.

In normal cells, where pairing and coiling are complete, the chiasmata are situated near the chromosome ends. Pairing must, therefore, start at the ends if, as has been suggested previously by Darlington (1935), the degree and distribution of pachytene pairing and coiling determine the number and position of chiasmata. It must then be assumed that, in the precentric cells of *C. atratum*, the paired chromosomes usually pair and coil at only one end and consequently only a single terminal chiasma is formed.

3. THE ORIGIN OF THE ABNORMALITY

These peculiarities of meiosis arise, as we should expect, before first metaphase. The precentric cells come into prophase later, for while normal cells are at diplotene the abnormal cells are still in a pre-pachytene condition. Later they catch up and both types of

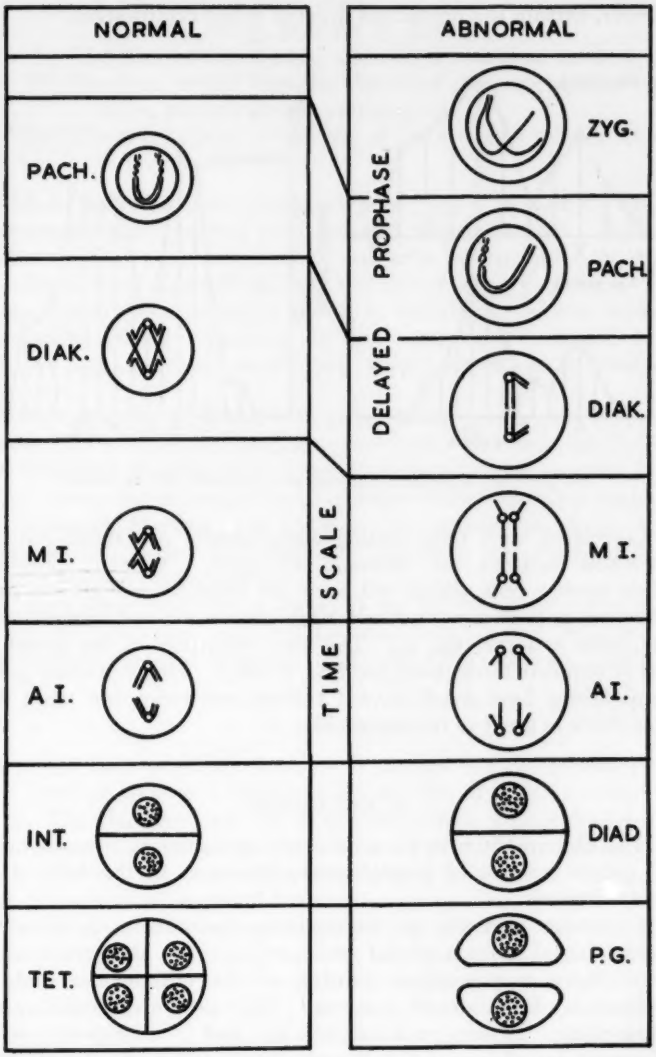

FIG. 4.—The two types of meiosis in pollen mother cells of *C. atratum*. The reduced pairing and precociously split centromeres in precentric cells results in the formation of dyads.

cell enter first metaphase together (fig. 4). Pachytene and later prophase stages must, therefore, progress more rapidly than in normal cells. Chromosome pairing and relational coiling in the precentric cells is, it is assumed, less complete than in the normal cells. This will result in a reduced chiasma frequency and, if coiling starts at the chromosome ends, more complete terminalisation. It will not, however, explain the precocious division of the centromeres.

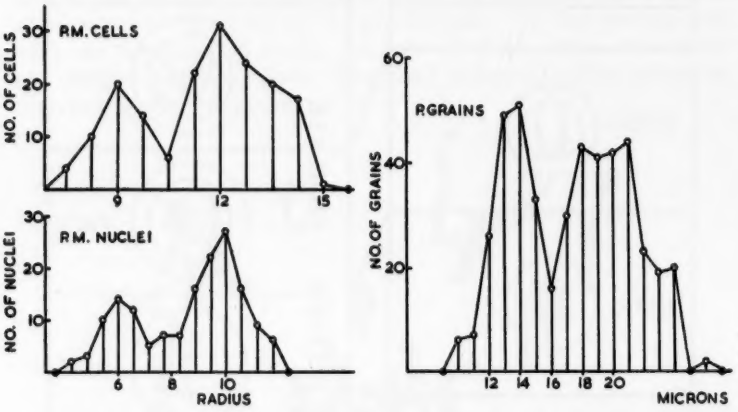

FIG. 5.—The relative sizes of normal and precentric cells and nuclei.

Correlated with these timing abnormalities are significant size differences between the different cell types. At early prophase, pollen mother cells which will show an irregular meiosis are more than twice as large as normal cells while their nuclei are approximately four times as large (fig. 5). This size advantage of the precentric cells is retained throughout the first division. Hence mature pollen grains arising from dyads have a volume, not twice, but more than three times as great as normal grains.

4. DISCUSSION

The abnormalities in *C. atratum* are, presumably, brought on by the genetic control of physiological differences in the cells at the onset of meiosis.

C. atratum and the parthenogenetic earthworms described by Muldal (1952), form a special group, in regard to abnormal meiosis, where there is precocious splitting of the centromeres and the production of unreduced gametes. This precentric behaviour is intermediate between normal meiosis and "asynapsis" where chiasmata are absent and only univalents are found. In all other respects the abnormality in *C. atratum* is analagous to that found in

Antennaria (Stebbins, 1932), *Hieracium* (Gustafsson, 1942), *Calamagrostis* (Nygren, 1946) and *Scilla* (Rees, 1952).

The similarities are :—

(i) Only some germ cells, not all, in each anther are affected. The proportion of abnormal cells varies from as little as 1 per cent. in some samples of *Scilla* to 63 per cent. in *C. atratum* and 99 per cent. in *Datura*.
(ii) The distribution of abnormal cells is always at random.
(iii) Prophase begins later in abnormal cells, metaphase sometimes, but not always, catching up.
(iv) There is increase in the size of the retarded cells in relation to the normal.

Male sterile *Lathyrus odoratus* (Upcott, 1937) is, like *C. atratum*, also intermediate between asynapsis and normal meiosis. It is also similar in that prophase in abnormal cells begins later. It differs in being a total defect of all cells and in not being precentric. The delay in early meiosis brings about, in this case, a greater terminal localisation of the chiasmata at first metaphase. Carried to its extreme this increased localisation would result, as in *Scilla*, in asynapsis.

The behaviour of E.M.C's. in these various plants, may be similar to, or different from, the pollen mother cells. The cells arising from the abnormal divisions may either give rise to fertile gametes or, in other cases, degenerate at varying stages before reaching maturity. In *Antennaria*, *Hieracium* and *Calamagrostis*, meiosis may follow yet a third course : it may be replaced by mitosis in the abnormal pollen mother cells and embryo sacs.

Many other examples of asynapsis are known at meiosis in plants where the properties that are discussed here are not described, as in *Datura* (Satina and Blakeslee, 1934) and *Pyrus* (Thomas, 1942).

5. SUMMARY

1. An autotetraploid clone of *C. atratum* has unreduced pollen as a result of abnormal meiosis in 68 per cent. of pollen mother cells.

2. The abnormal cells lag at prophase, have a prolonged growth phase, and are less precocious in chromosome development than normal cells. They are consequently larger in both cell and nuclear size throughout all stages of division.

3. In these cells no quadrivalents are formed. The bivalents show a reduced chiasma frequency and precocious splitting of the centromeres at MI. The chromatids fall apart during anaphase.

4. The first division gives rise to cells with 36 chromosomes. A second division is omitted. Thus, it appears, it is the capacity of the centromeres for division which determines or precipitates the occurrence of the second meiotic division.

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Plate

1. Normal first metaphase with four quadrivalents and ten bivalents. ×2600.
2. First metaphase in precentric cell with eighteen bivalents and precociously divided centromeres. ×2600.
3. Two normal first anaphases with eighteen daughter bivalents in each. ×1000.
4. First anaphase in a precentric cell; 36 chromosomes passing to each pole. ×1000.

THE GIANT SEX CHROMOSOMES IN THE MAMMAL *MICROTUS AGRESTIS*

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I. INTRODUCTION

THE study of mammalian chromosomes, despite their importance, has been greatly neglected. This is in part due to the idea that they are unsuitable for a proper cytological study (*e.g.* White, 1949). The best mammalian chromosomes are to be found in the marsupials, many of which have a small number of large chromosomes. Most other mammals on the other hand, have a large number of small chromosomes so that an accurate study is certainly a more difficult task than in many other organisms. But the extreme difficulties which have often been attributed to the study of mammalian chromosomes are also in part due to a lack of technique. This has led to confusion on even such matters as the chromosome number of a particular species (Sachs, 1952*b*) and it now seems desirable to re-investigate much of the earlier work using more suitable techniques.

One of the many interesting points in mammals is that the animals have sex chromosomes which may be morphologically distinguished from the autosomes. These sex chromosomes are of importance both from a genetical and from a cytological point of view. Their genetical interest is connected with the existence of complete and partial sex linkage and their cytological interest is connected with their special behaviour during mitosis and meiosis. Some species of mammals show little or no size differences between the sex chromosomes and the autosomes. In these cases, therefore, identification of the sex chromosomes has not been possible at all the stages of division. But in *Microtus agrestis*, the short-tailed bank vole, the sex chromosomes are enormous and there is no difficulty in distinguishing them at mitosis and meiosis. An investigation of these giant sex chromosomes has been one of the purposes of the present study.

The existence of giant sex chromosomes in Swiss animals of *M. agrestis* had previously been reported by Matthey (1950) whose study was almost entirely based upon sectioned material. It has, however, now become possible, by the use of suitable squash techniques, to obtain much additional information not only on the sex chromosomes but also on general problems of chromosome behaviour and the sequence of meiosis.

2. MATERIAL AND TECHNIQUES

The animals used in the present study were derived from two sources. Some were caught on the grounds of Bayfordbury, and others were obtained from Dr D. Chitty of Oxford. Animals from the two locations gave the same results.

The main technique was fixation in 1 : 3 acetic-alcohol followed by Feulgen staining and squashing (Sachs, 1952*b*) and this gave uniformly good results. In addition, fixation in aceto-carmine or acetic acid followed by the normal method of Feulgen staining, and fixation in 1 : 3 acetic-alcohol with a small amount of ferric chloride followed by aceto-carmine staining, also proved useful. As is shown by their own photographs, the staining procedures for mammalian testes recommended by Slizynski (1949) and Makino and Nishimura (1952) show no observable advantage over the normal method of making Feulgen squashes. This also applies to the section cutting method which is used by Matthey (1950).

3. HETEROCHROMATIN AND THE SEX CHROMOSOMES

The English animals of *M. agrestis* from Hertfordshire and Oxfordshire, like the Swiss animals studied by Matthey (1950) have a chromosome number of $2n = 50$. This number and the giant sex chromosomes thus appear to be characteristic for the species. The giant sex chromosomes can be easily identified in spermatogonial mitosis. The size of each chromosome, however, varies according to stage and fixation, so that it is useless to make absolute chromosome measurements. At mitotic metaphase, however, after acetic-alcohol fixation and Feulgen staining, the autosomes are about 1-2μ long whereas the X chromosome is about 9μ and the Y chromosome about 6μ long (plate I, fig. 5).

Spermatogonial cells of the testes show that the sex chromosomes contain heterochromatin and this can be seen in some resting nuclei as two distinct chromocenters. But other resting nuclei, in different tissues, show no chromocenters. The detection of heterochromatin in the sex chromosomes by their appearance as chromocenters is thus not possible in all types of cells.

This presence or absence of chromocenters in the so-called resting nucleus is probably due to the different metabolic activities of the cells concerned. That the cytological appearance of heterochromatin can be modified by different metabolic activities of the cell was first found in studies on the effects of low temperature (Darlington and La Cour, 1938, 1940, 1941 ; La Cour 1951, *et al.*). This has shown that heterochromatin can, in certain species, show different differential behaviour under different environmental conditions. Heterochromatin which was intensely stained in resting nuclei could, after the effects of low temperature, be less intensely stained than the euchromatin at metaphase. Thus, as Darlington (1947) has pointed out, this behaviour of heterochromatin can be explained by the presence of genes which show allocycly under certain conditions. It can now be seen from the giant sex chromosomes that under some conditions the heterochromatin completely loses its differential stainability even in the resting nucleus.

The allocyclic behaviour of heterochromatin, including the loss of differential stainability, can also be seen in the behaviour of the X chromosomes in some grasshoppers and crickets (White, 1940). In these insects the X chromosome shows what White calls " negative heteropycnosis " during the early spermatogonial divisions ; later it loses its differential stainability ; and it becomes " positively heteropycnotic " during the prophase of meiosis. This behaviour of the X chromosome can also be related to the different appearance of heterochromatin under the differing conditions of the cells. Thus, in some cells, the heterochromatic genes react in the same way as the euchromatic genes and cannot be cytologically detected. In other cells, with the appropriate metabolic conditions, they can be detected by their differential metabolic cycle and a collection of such neighbouring genes can then be seen as distinct blocks of heterochromatin. So it is that, although some resting nuclei have no chromocenters, the appropriate conditions show that the X and the Y in *M. agrestis* contain heterochromatin.

4. THE SEX CHROMOSOMES AT MITOSIS

In spermatogonial cells the sex chromosomes at mitosis are, in relation to the autosomes, precocious during prophase and they lag during anaphase. But at mitotic metaphase both the sex chromosomes and the autosomes are arranged upon the spindle. During anaphase the X and the Y start to separate from the centromeres, but they are still held together when the autosomes have completely separated to the two poles (plate I, figs. 4 and 6). This slower separation of both X and Y may be in part due to the large size of these chromosomes moving in a restricted space. But it is more probably also due to the heterochromatin holding together the two chromatids.

In spermatogonial cells the spiral structure of the sex chromosomes is especially clear during the early prophase in the pre-meiotic mitoses. What appears to be an occasional case of somatic pairing, between a small segment of the X and a small segment of the Y, has also been seen at this stage (plate I, fig. 2).

5. TERMINAL CENTROMERES

The mitotic cells in the ovary show that the female also have two enormous sex chromosomes, and that they both have submedian centromeres. The giant chromosomes with submedian centromeres are, therefore, the X chromosomes. The position of the centromere in the X and Y of the male has been most clearly seen at spermatogonial mitosis (plate I, figs. 3 and 5). The anaphase figures in these cells confirms that the X has a submedian centromere whereas the centromere of the Y appears to be terminal. Examination at mitotic metaphase again confirms the submedian centromere of the X but it also shows that the Y has, in addition to the long arm, a small

structure at the centromere end of the chromosome. This does not, however, necessarily imply (*cf.* White, 1951) that telocentrics in reality have a small second arm.

The existence of stable telocentrics, *i.e.* chromosomes with stable terminal centromeres, has often been doubted (Matthey, 1951*a*; White, 1951). This is partly due to the fact that the newly-formed telocentrics studies in plants such as *Zea*, *Tulipa* and *Fritillaria*, were found to be unstable. But this unstable condition does not apply to all telocentrics. Thus Darlington and La Cour (1950) have found that telocentrics in plants of *Campanula* may be unstable or stable, and that stable telocentrics can maintain themselves in nature. A further point which is often overlooked by those who doubt the existence of stable telocentrics, is that the centromere is not just a gap or a single point in the chromosome. The centromere is a multiple structure which can be broken at different places (Darlington, 1939). The functional telocentric will thus still possess at least some and probably all of this centromere structure so that the centromere end of the telocentric is not just an abrupt end to the chromosome arm. The existence of this centromere structure in telocentrics has in fact been seen in plants of *Phleum* (Ellerström and Tjio, 1950). The Y in *M. agrestis* may also be a telocentric showing this centromere structure at metaphase. It can thus be concluded that stable telocentrics can exist, and their existence must be reckoned as a factor in chromosome evolution.

6. A SEX VESICLE AT MEIOSIS

An examination of testes containing cells at meiosis shows that the autosomes in many of the cells are at pachytene. A most striking first impression of these cells is that the nucleus contains, in addition to the pachytene autosomes, a large oblong body which is strongly Feulgen positive. Closer examination shows that this large Feulgen positive body consists of two Feulgen positive chromosomes, the X and the Y, surrounded by a somewhat lighter stained vesicle (fig. 1C, and plate II, fig. 4). Thus when the autosomes are at pachytene, the X and the Y are included in a special vesicle which can be designated as a sex vesicle. The existence of a sex vesicle surrounding the giant sex chromosomes in another species of *Microtus* has been reported by Muldal (1949).

The development of this sex vesicle in *M. agrestis* has already begun when the autosomes are at leptotene. At this stage the sex chromosomes are still separate, the X and the Y each forming what appears to be its own vesicle. The X and the Y then move together until they are included in the one large vesicle at pachytene (plate II, figs. 1, 2 and 4). The vesicle surrounding the sex chromosomes still persists at diplotene (fig. 1A and B) and diakinesis, but it has in most cases disappeared when the autosomes reach 1st metaphase. The

formation of this sex vesicle is characteristic of meiosis and it has never been seen at mitosis.

The sex chromosomes are thus inside their vesicle during those stages of meiosis where chiasmata are normally formed. This raises

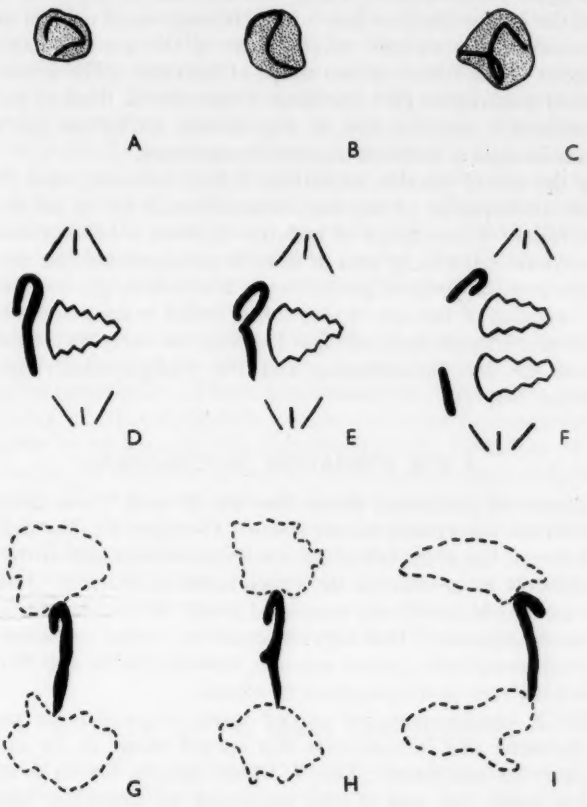


FIG. 1

A and *B*. The X and Y inside the sex vesicle ×2000 at diplotene.
C. The X and Y inside the sex vesicle at pachytene.
D and *E*. The X and Y connected at 1st metaphase.
F. The X and Y not connected at early 1st anaphase.
G, *H* and *I*. The X and Y connected and lagging at late 1st anaphase.

N.B.—The arrangement of the sex chromosomes inside the sex vesicle is comparable to their arrangement when the sex vesicle has disappeared.
In *D*, *E* and *F* autosomes and spindle are drawn diagrammatically.

the question whether chiasma formation between the X and the Y in *M. agrestis* can and actually does take place. Matthey (1950), on the basis of his observations at 1st metaphase, at first concluded that chiasmata had been formed between the X and Y in *M. agrestis*.

Later, however (1951*a*), he concluded that this was doubtful. Both these opposing conclusions were based on the same set of observations. This confusion is probably caused by Matthey's own observations on what he supposed to be the meiotic development of the sex chromosomes *before 1st metaphase*. These had led him to conclude that the X and the Y flow together into a large homogeneous mass of material. He considers this meiotic development of the sex chromosomes as analogous to the fusion of two drops of mercury. The union of two masses of material to give one large homogeneous mass at pachytene thus seemed a contradiction to any normal pachytene pairing and chiasma formation between the sex chromosomes.

By the use of suitable techniques I find, however, that the early meiotic development of the sex chromosomes is not at all analogous to the fusion of two drops of mercury to form a homogeneous mass at pachytene. It can be seen in suitable preparations that the oblong Feulgen-positive body at pachytene is not a homogeneous structure, that it consists of two sex chromosomes inside a sex vesicle, and that there is no inherent contradiction between the early meiotic development of the sex chromosomes and the configurations seen at 1st metaphase (fig. 1).

7. THE FORMATION OF CHIASMATA

A study of pachytene shows that the X and Y are arranged in characteristic ways inside the sex vesicle. Owing to the faint differentiation between the materials of the sex chromosomes and of the vesicle, it is difficult to be sure of the exact mode of pairing. It appears, however, that X and Y are connected inside the vesicle end to end or by a small segment. This segment could be about the same size as the small somatically paired segment between the X and Y which is occasionally seen at the prophase of mitosis.

The X chromosome lies curved inside the vesicle at pachytene and diplotene and it maintains this curved shape at 1st metaphase and even 1st anaphase. The Y which usually lies in a straighter position inside the vesicle also maintains its straighter position at 1st metaphase and anaphase. The sex vesicle often lies on one side of the nucleus and the sex chromosomes at 1st metaphase are often on the edge of the plate.

The pachytene and 1st metaphase configurations can both be explained by the existence of a small pairing segment between the X and the Y. The configurations at 1st metaphase and 1st anaphase show that this pairing segment would be at the end furthest from the centromere in both chromosomes, and (as is to be expected) only reductional separation of the sex chromosomes occurs at 1st anaphase.

The X and Y chromosomes are not always connected at 1st metaphase and in these cells the sex chromosomes have presumably been unpaired.

Darlington (1934) was the first to suggest a cytological explanation which could account for complete and incomplete sex linkage in mammals. This showed that both types of sex linkage could be explained by the existence of a pairing segment and a differential (non-pairing) segment in the X and the Y. Genes on the differential segment would be completely sex-linked. Genes on the pairing segment would be partially sex-linked by the formation of chiasmata within the pairing segments. In the absence of any knowledge of the genetics of *M. agrestis*, nothing can be said about the presence or absence of incomplete sex-linkage in this animal. But from the cytological point of view the pachytene, 1st metaphase and 1st anaphase configurations can all be explained if the sex chromosomes have a small pairing and large differential segments. Chiasmata can then be formed within the pairing segment of the X and the Y. Remnants of the sex vesicle may, however, still be present after diakinesis. This could conceal the chiasmata at 1st metaphase and cause the sex chromosomes to lag at 1st anaphase.

The presence of a sex vesicle during the prophase of meiosis which is so prominently seen in *M. agrestis*, is not a unique phenomenon restricted to this species. I have seen similarities to this type of meiosis in a number of other mammals. As genes for sex determination are presumably located on the sex chromosomes, the formation of a sex vesicle during the prophase of meiosis could have distinct evolutionary advantages. The sex vesicle prevents any possibility of crossing-over between the sex chromosomes and the autosomes which might otherwise arise owing to errors of pairing at pachytene. The sex vesicle could thus ensure that these sex-determining genes are always maintained on the sex chromosomes. This mechanism could then prevent the occurrence of gene combinations which might upset the normal balance for sex determination.

8. ORIGIN OF THE GIANT CHROMOSOMES

There is very little cytological data on the chromosomes of other species in the genus *Microtus*. Out of more than 200 different forms, only about half a dozen have been cytologically examined. Even among these there is some disagreement as to the chromosome number and the presence of giant sex chromosomes. It is, however, clear that species with higher chromosome numbers than *M. agrestis* have been found in *Microtus* and in the allied genus *Clethrionomys*. Thus *M. nivalis* (Matthey, 1951*b*) and *C. glareolus* (Sachs, 1952*b*) both have $2n = 56$ and no giant sex chromosomes.

M. agrestis with $2n = 50$ and giant sex chromosomes could have originated from these or similar species with higher chromosome numbers. Thus in the plant *Phyllospadix japonica*, Harada (1944) reports that the female plants have 20 small chromosomes and the male plants have 15 small and one enormous chromosome. This

large chromosome contains heterochromatin, is designated as the Y chromosome, and appears to have originated from the combination of 5 short chromosomes.

9. LAMPBRUSH CHROMOSOMES AND THE SEQUENCE OF MEIOSIS

Meiosis in the male of *M. agrestis* includes a modified form of what appears to be diplotene. At this stage the autosomes have a "woolly" outline with small protrusions connected to the chromosomes (plate II, fig. 3). This type of diplotene, described as diffuse diplotene, has been observed in a number of plants and animals (Darlington, 1937). Its most extreme development has been found in animal oocytes, where the chromosomes are referred to as lampbrush chromosomes. There is a distinct similarity between these and what can be called the lampbrush chromosomes in *M. agrestis*. In *M. agrestis* the lampbrush structures begin to develop at pachytene and show their maximum development at what is apparently diplotene. In the animal oocytes there is also a maximum development of the lampbrush at what is apparently diplotene (Dodson, 1948).

It can be shown that lampbrush development is associated with chemical changes in the chromosomes. For example, in the oocytes development of the lampbrush chromosomes is associated with a decrease in Feulgen staining. This decrease is associated with an increase in length of the chromosomes and in fully grown oocytes the chromosomes, except in a few small regions, are completely Feulgen negative (Serra, 1947). The change from a Feulgen-positive to a Feulgen-negative chromosome associated with this extreme lampbrush development may be due (*a*) to the increased growth of the chromosomes with the same amount of DNA (desoxyribose nucleic acid) decreasing the intensity of Feulgen staining and/or (*b*) to the transfer of DNA from the chromosomes.

Ris (1951) maintains the former explanation and denies the existence of DNA transfer from the chromosomes. There are, however, several lines of evidence which show that nucleic acid transfer does take place. Thus, the lampbrush development in *M. agrestis* is not associated with an increased size of the chromosomes. In this species the chromosomes at diplotene are if anything shorter than those at preceding stages of meiosis. The same applies to the lampbrush stages in grasshoppers (Srivastava, 1951).

Evidence for the existence of nucleic acid transfer can also be obtained from studies on mitosis. Howard and Pelc (1951) have concluded that DNA is synthesised during the resting stage in cells which are going to divide. Distinct Feulgen-positive chromosomes are however only found at prophase, so that it is reasonable to assume that the DNA synthesised during the resting stage is transferred to the chromosomes at prophase. In the same way DNA can be trans-

ferred from the chromosomes at telophase when the chromosomes lose their distinct Feulgen positive structure. The transfer of DNA to the chromosomes can also be inferred from results on the effects of low temperature (Darlington, 1947).

Studies on RNA (ribose nucleic acid) have likewise shown the transfer of RNA to and from the chromosomes. Thus Jacobson and Webb (1952), who assume that nucleic acid in the cell always occurs in conjunction with a protein, conclude that ribonucleoprotein is transferred to the chromosomes during prophase and transferred from the chromosomes during anaphase. This latter process can be inferred by the presence of the transferred ribonucleoprotein between the two groups of anaphase chromosomes. These different lines of approach all show that nucleic acid transfer to and from the chromosomes can and does take place.

The synthetic processes of mitotic cells, such as protein synthesis, take place during the resting stage and it can be seen that they are preceded or associated with the transfer of nucleic acid from the chromosomes. Those stages of meiosis which show evidence of nucleic acid transfer from the chromosomes, as in the case in the formation of lampbrush chromosomes, are thus in this respect analogous to the synthetic part of the resting stage in mitosis. This analogy between lampbrush and resting stages can further be seen in the behaviour of heterochromatin. It has been shown that the sex chromosomes in *M. agrestis* contain heterochromatin which can be seen as chromocenters in some resting nuclei. These parts of the chromosomes therefore can maintain their DNA content even in these resting stages. In the same way the sex vesicle at diplotene, which contains the sex chromosomes, does not show the lampbrush formation which is shown by the autosomes : the heterochromatin during this stage of meiosis maintains its DNA content. The same differential behaviour of heterochromatin has thus been found both in resting stages of mitosis and in lampbrush stages of meiosis, and this also emphasises the similarity of these two stages. Lampbrush formation during certain stages of meiosis may thus be considered as a sign of synthetic processes inside the cell.

The existence of synthetic processes after chromosome pairing enables us to extend our knowledge of the sequence of meiosis. It has been shown in a number of organisms that the beginning of meiosis is connected with special circumstances. These include the supply of materials from tissues surrounding the germ cells, and the existence of special types of mitosis before meiosis. The supply of materials from tissues surrounding the germ cells (Darlington and La Cour, 1946 ; Painter, 1943 ; Montalenti *et al.*, 1950) includes the possibility of nucleic acid secretion associated with the beginning of meiosis. The direct evidence for special types of mitosis before meiosis comes from *M. agrestis* and some insects (White, 1940) which show that meiosis is preceded by rapid mitotic cycles. In these

insects it has been seen that there is almost no resting stage between the pre-meiotic mitoses. There is thus not a complete DNA transfer from the chromosomes during telophase so that the autosomes have an irregular outline at the beginning of the next prophase. This irregular outline is also found in the autosomes of *M. agrestis*, but it is not shown by the sex chromosomes which can maintain their DNA content even during resting stages. The special character of pre-meiotic mitoses can also be inferred from the existence of mitotic abnormalities which only occur before meiosis (Sachs, 1952*a*).

The sequence of events leading up to meiosis can explain the presence of lampbrush chromosomes. It can reasonably be assumed that the rapidity of mitosis before meiosis excludes synthetic processes which would also be sufficient for chromosome reproduction during meiosis. The supply of substances from outside the germ cells may allow these cells to start on meiosis and proceed up to pachytene. Judging from the large proportion of cells at pachytene in the spermatocytes of *M. agrestis*, the cells remain at this stage for a long time. The continuation of meiosis is however dependent upon the synthesis of new chromosome material : this would take place after chromosome pairing and be made apparent by the lampbrush chromosomes.

The existence of this synthetic stage after chromosome pairing at meiosis allows us to understand one of the fundamental differences between mitosis and meiosis. This difference lies in the time of chromosome duplication (Darlington, 1937). Chromosome duplication at mitosis takes place at the resting stage and chromosome duplication at meiosis takes place after chromosome pairing at pachytene. It can now be seen that the resting stage of mitosis and these lampbrush stages after pairing at meiosis can both be considered as stages for the synthesis of chromosome material. The differences in the times of chromosome reproduction at mitosis and meiosis may thus be due to the differences in the time of synthesis of chromosome material.

10. SUMMARY

1. The rodent *Microtus agrestis*, with $2n = 50$, has enormous sex chromosomes which can be easily identified at mitosis and meiosis. The males have an X Y sex mechanism.

2. Both X and Y contain (or entirely consist of) heterochromatin which appear as chromocenters in *some* cells. This variation in the appearance of heterochromatin is probably due to different metabolic activities in the different cells.

3. At spermatogonial mitosis the sex chromosomes are precocious during prophase and lag during anaphase. There is occasional somatic pairing at mitotic prophase between small almost terminal segments of X and Y.

4. The X and Y are separate at the beginning of meiosis and are included in a special sex vesicle by pachytene. The arrangements

Plate I

Microphotographs of mitosis in the testes of *Microtus agrestis*. Feulgen × 3000.

FIG. 1.—Early Prophase. Early development of the X and Y.

FIG. 2.—Later prophase. Somatic pairing between a small segment of the X and Y.

FIGS. 3 and 5.—Metaphase.

FIG. 4.—Early anaphase. X and Y beginning to separate.

FIG. 6.—Late anaphase. Delayed separation of the X and Y.

Plate II

Microphotographs of meiosis in the testes of *Microtus agrestis*. Feulgen ×2000.

FIG. 1.—Stages in the development of the sex vesicle. The X and Y still separate (left hand cell); lying next to one another (centre); nearly included in the one sex vesicle (right hand cell).

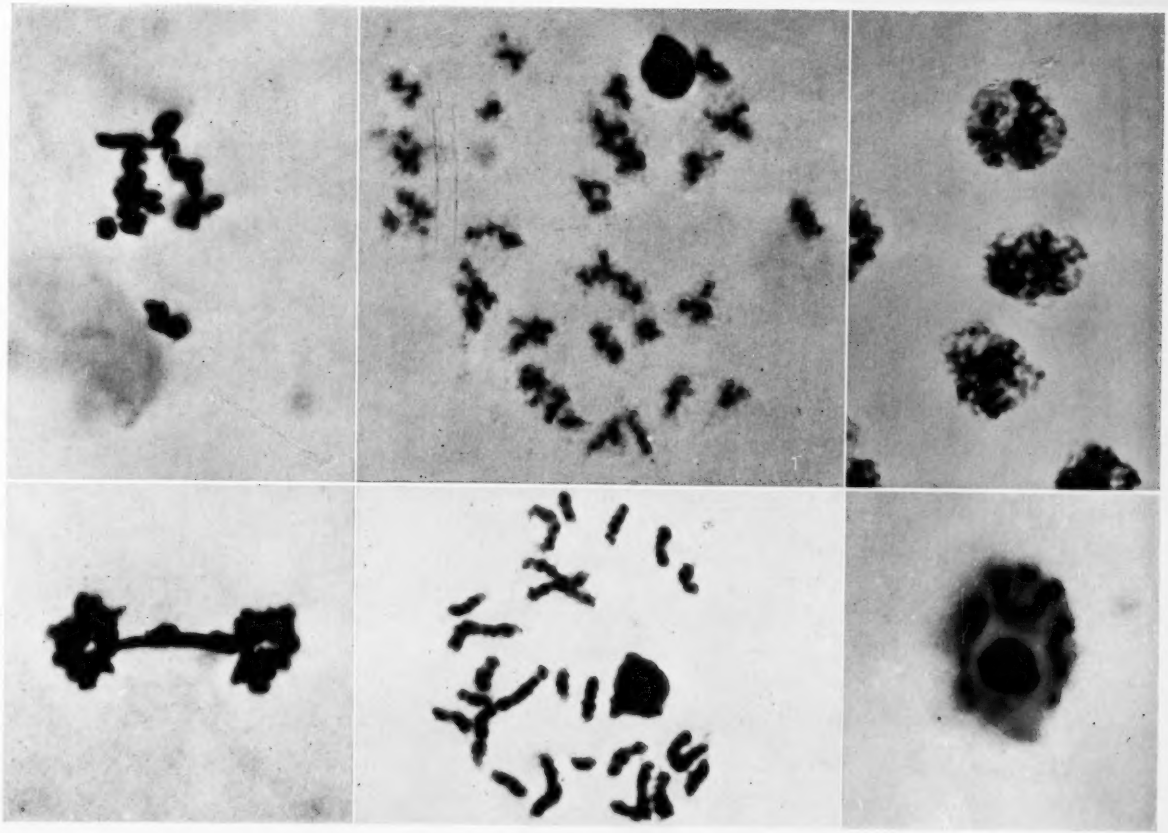
FIG. 2.—Sex vesicle at pachytene. Unsquashed nucleus.

FIG. 3.—Sex vesicle at diplotene. Lampbrush autosomes.

FIG. 4.—Sex vesicle at pachytene. Squashed nucleus.

FIG. 5.—Early anaphase I. Curved arrangement of the X and straighter position of the Y.

FIG. 6.—Late anaphase I. Lagging of X and Y.



of the sex chromosomes inside the sex vesicle are comparable to their arrangement at 1st metaphase when the vesicle has usually disappeared.

5. The configurations of the sex chromosomes from pachytene to 1st anaphase can be explained by the existence of a small pairing and large differential segments. The pairing segments in both X and Y would then be at the ends furthest from the centromere.

6. *M. agrestis* and its giant sex chromosomes could have originated from related species with higher chromosome numbers and no giant sex chromosomes.

7. Meiosis in the male *M. agrestis* includes stages with lampbrush chromosomes showing similarities to resting stages between mitoses. The development of lampbrush chromosomes at meiosis is interpreted as a sign of synthesis of chromosome material. Differences in the time of chromosome duplication at mitosis and meiosis can thus be explained by differences in the time of synthesis of chromosome material.

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THE GRAPHIC EVALUATION OF A BREEDING SYSTEM

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I. INTRODUCTION

MAIZE varieties in the field vary in their self-pollination. This indicates that control of the inbreeding-outbreeding mechanism is not so simple as is usually supposed. Usually a population's mean outcrossing or selfing has been recorded, with neglect of plant to plant variation. Behaviour of individual plants within a population has not been considered. The variation has been reduced to a mean which conceals or rejects some of the most useful information.

Maize is a *monoecious* (with male and female flowers separate on the same plant) outcrossing species with the possibility of inbreeding. The table gives the average inbreeding for different populations under different environments. These values, however, depend on similarities between the tested and tester variety for characters, such as relative flowering times, affecting dissemination and reception of pollen.

Jones and Brooks (1950) have listed nine factors affecting natural outcrossing in cross-pollinated grasses. They have omitted protandry and protogyny, and also plant to plant variation in flowering time, presumably assuming these to be of little interest. Yet these properties must be of cardinal importance for the breeding system of maize. Even extensive contamination studies have failed to determine the amount of inbreeding and outbreeding between and within plants of one variety.

Recently I have made a multiple regression analysis on *silking time* (protrusion of styles), *tasselling time* (opening of anthers with consequent shedding of pollen) and *dichogamy*, or the difference of the two in time, in order to determine their influences on out-crossing of sweet corn inter-planted with a flint variety. All effected contamination (Haskell, 1953). However, besides knowing behaviour of varieties as a whole, knowledge of individual behaviour within each is also valuable, as dichogamy relations of the breeding system are important in the evolution of species (*cf.* Darwin, 1888). A graphic method, suggested by Dr Darlington, is given here of rapidly evaluating individual behaviour within a population.

Dichogamy consists of *protandry* and *protogyny*, each of which may be partial or absolute: absolute protandry is when stamens are ripe and all pollen has been shed before the stigmas are receptive, so that no self-pollination occurs within the flower; partial protandry

is when much of the pollen is shed prior to the styles ripening, but there is some overlapping so that some of the earliest styles may receive pollen shed by late anthers. Protogyny is equivalent to negative protandry : with absolute protogyny the styles and stigmas are receptive and are already fertilised, or inactivated, before pollen starts to shed ; or some of the styles are still receptive when the pollen is being liberated (partial protogyny).

There is a further complication in maize that the ears, really female inflorescences, do not open at the same time : the main ear usually opens ahead of the secondaries. In the *flint* types of both maize and sweet corn, with which we are here dealing, one ear alone predominates. In *dent* types there are several ears opening consecutively, so that the amount of inbreeding or outbreeding is further complicated, being dependant on the position of the female inflorescence on the plant.

2. EXPERIMENTAL DATA

Professor K. Mather's data were used. These recorded first pollen shedding, and date silks first appeared on main ears of plants in two maize varieties, a flint and sweet corn, sown 24th May 1945. Fifty plants of each were raised at random in two adjacent plots. Data from both were pooled. There were altogether 94 flints and 87 sweets, as a few plants died.

TABLE

Self-fertilisation in maize populations

Comment	Selfing	Author
Dent corn in Nebraska. Both varieties with the same flowering periods	0·70 per cent.	Kiesselbach, 1922
Dent corn in Maryland. Average of five years	3·70 ,, 5·00 ,,	Kemp and Rothgeb, 1943 Hayes, 1918
Maize crops generally throughout U.S.A.	5·00 ,, 5·13 ,,	Wallace and Bressman, 1949 Waller, 1917

Figures represent means of isolated plants surrounded by plants of another variety. Outcrossing was detected with marker genes, e.g. *Y-y*.

3. THE TWO-DIMENSIONAL GRAPH

Fig. 1 gives a conventional plant breeding method of estimating earliness and uniformity. The percentage of flints and sweets commencing to flower during July are given, with silking and tasselling plotted separately. The graph indicates broadly that flints silked considerably earlier than they tasselled ; the crop burst into pollen shedding. A few later flowering stragglers are depicted along the

bottom right of the graph. The sweet variety gradually came into flower, and was somewhat later than the flint. Several late silkers are also shown.

This method has several disadvantages. Sunday measurements are absent, so introducing irregularities. More important, silking and tasselling relations of individuals within a variety are not shown.

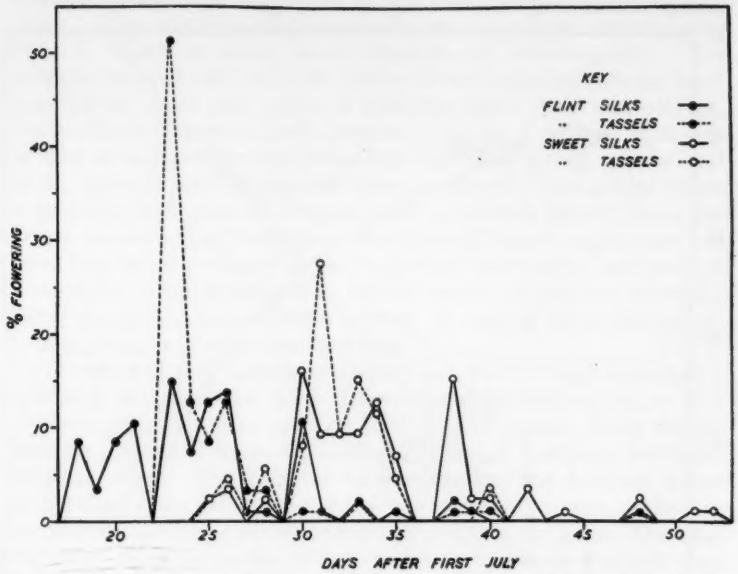

FIG. 1.—Conventional two-dimensional method of plotting earliness and uniformity. Open circles refer to sweet corn plants ; black circles refer to flinty maize.

4. THE THREE-DIMENSIONAL GRAPH

The same data are plotted in fig. 2. An arithmetical scale replaces actual flowering date in July. Silking date is plotted along the Y-axis, and tasselling date along the X-axis. Individual behaviour can now be charted ; as several plants were alike, circles of relative sizes are used to indicate how many have the same flowering relations. Percentages need not be calculated. Full and open circles respectively indicate flint and sweet corns. Circles on the dotted line represent plants silking and tasselling on the same day. Circles below the line are protogynous plants, while those above are protandrous ; distance from the line is proportional to the amount of protandry or protogyny.

The breeding behaviour in both populations is now readily evaluated. The earliest flints are highly protogynous and represent a large proportion of the population. Slightly later ones are more homogamous. Latest ones tend to be more and more protandrous ;

however, some of the last flints to flower are also highly protogynous. The earliest sweet corn plants show little dichogamy but within the population there is a definite trend : later plants have a greater tendency to be protandrous. Only a few plants in the middle flowering period of the populations are protogynous. The best fitting straight line for the sweet corn data was calculated. This was $Y = 1{\cdot}16X - 2{\cdot}71$, and is plotted in fig. 2.

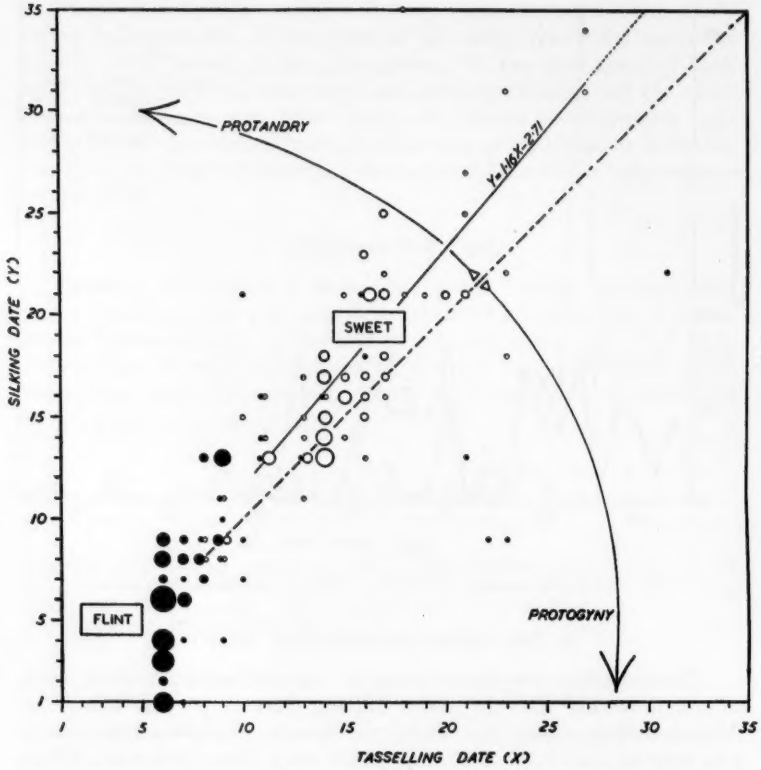

FIG. 2.—Three-dimensional method of plotting earliness and uniformity. Open circles refer to sweet corn plants ; black circles refer to flinty maize. Size of circles is proportional to number of plants. $Y = 1{\cdot}16X - 2{\cdot}71$ is the best fitting straight line for sweet corn values.

5. FLOWERING TIME AND THE BREEDING SYSTEM

Fig. 1 describes population behaviour without considering the *individuals* within it. It assumes that these are generally the same, except for their time of flowering. The breeding system of two populations in the same environment is also described in fig. 2, using a three-dimensional graph. This provides information not previously apparent.

The amount of selfing in a plant depends on availability of its own pollen. Hence earliest sweet corn plants have less chance of selfing, as they have available a pollen mixture before and after tasselling commences. Outbreeding is also more likely to be higher when plants are protogynous : this is mostly among earliest plants in both populations. Seeds from the earlier portion of the population, therefore, possibly include more heterozygotes than those from later plants. More vigorous offspring would thus come from earlier plants, because vigour in maize partly depends on heterozygosity. The stragglers are not only later, but pollen choice is limited. Being more protandrous, their own pollen is shedding when their silks emerge. Latest plants therefore have greater chances of selfing, especially as silks do not emerge simultaneously but often spread over several days. More of their seeds would then be inbred. Now inbred maize is generally later than the original open pollinated parent, hence the next generation from these seeds would be still later. Again they are more likely to be protandrous and to inbreed both within and between themselves. Thus, genetically, a vicious circle is set up, so that besides being temporally isolated from the bulk of most of the crop's pollen, homozygosity is even further forced on them.

Duration of pollen shedding, which has not yet been recorded in England, influences the inbreeding-outbreeding mechanism, so that absolute protandry does not actually occur in maize. Even though absolute protandry discourages self-pollination, it is still not fool-proof (Mather, 1940). The floral mechanism of maize has, however, arisen by artificial selection and is a particular condition : here, protandry has not been so important as sex separation within the plant. Duration of pollen shedding in the U.S.A. varies from two to fourteen days according to variety and most silks under favourable conditions are pollinated on day of emergence with ears completely pollinated within three days (Kiesselbach, 1949). Hence all degrees of inbreeding and outbreeding are possible.

The genetical aspects of dichogamy in maize were earlier investigated by Kempton (1924). He found that although maize is protandrous with occasional exceptions, when a protogynous Spanish popcorn was crossed with a protandrous maize, the F1 were protandrous. The F2 segregated to give a greater mean protandry and a few protogynous plants. Kempton believed protogyny was a reversion to an ancestral condition, being normal for *Tripsacum* and *Euchlaena* ; it was probably not due to photoperiodism, but might have been a manifestation of male sterility which may postpone anther opening. No male sterility occurred in the flint variety used in the present experiment, so that male sterility is not a controlling factor here. Other cereals are also known in which the dichogamy relations vary according to the genotype : in rye, Vavilov (1951) found both cross- and self-pollinated forms, with it normally being regarded as outcrossing in Asia.

6. METHODS OF REPRESENTATION

Some consideration may now be given to other methods used by maize breeders for illustrating the flowering of maize. Shaw and Thom (1951) have plotted silking time-rate (days from 50 per cent. silking), positive or negative, against percentage of plants silked. In this way they compared a variety's rate of silking over several years. This method could be extended for studying environmental and other effects on flowering. Compared with fig. 2, it involves additional calculation of differences from the mean, and percentages. Yet it quickly demonstrated that for hot, dry years in the mid-West tassels rapidly appeared and had earlier pollen shedding, while there was a slower silking rate. Tassels appeared more slowly yet with more rapid silking in cooler, moister years. Thus, in the present data, climate might have influenced the earliest flint plants, which suddenly burst into pollen shedding. This is unlikely, however, since pollen shedding and silking for varieties sown at monthly intervals in England show high correlation regardless of climatic differences (Haskell, 1949*a*). That climate does influence the inbreeding-outbreeding system of some species is seen in wheat which is 99·9 per cent. self-pollinated in England, but shows increased outcrossing in drier climates (Vavilov, 1951).

When studying the effects of sowing date on the rate of sweet corn tasselling (1949*b*) I suggested a graph in which probit transformation of tasselling time is plotted against the logarithm of the flowering date. This helps to straighten the sigmoid-like curves so that uniformity and rate of tasselling can more readily be assessed. Cornfield (1949) objected to this on the grounds that probits could be used only for discrete values. The effect of the probit transformation, however, is to straighten sigmoid curves when ordinates are measured on a linear scale of probits instead of percentages (*cf.* Finney, 1947). Hence their use is legitimate here.

Another graphical way of displaying flowering time data is that of Singleton (1948) who graphed the percentage of silks which had emerged and of plants shedding pollen against date for four sowings of many sweet corn inbreds in Connecticut. Scaling was such that 100 per cent. silking equalled 50 per cent. shedding pollen ; hence critical sowing date for producing hybrid seed is rapidly determined. This allows a rapid visual test of whether two inbreds will flower together, important when planning cross-pollination for hybrid seed production. Singleton's graphs show that pollen shedding normally starts before silks appear. The 100 per cent. tasselling is always reached ahead of 100 per cent. silking, and pollen shedding always continues for several days after silking is completed. The present graph should similarly be useful for comparing the breeding behaviour not only of ordinary maize varieties, inbreds and single-cross and double-cross populations, but of other plant species.

7. SUMMARY

1. Individual silking date, time of first pollen shedding and number of plants of maize together plotted in a three dimensional graph give more information on the breeding system than the conventional plotting of percentage flowering against time.

2. The breeding behaviour of individuals within a population and between two inter-breeding populations is also readily assessed. Protandry in maize is not so important for out-breeding as being monoecious.

3. The relation of dichogamy with flowering time is also seen. Earliest plants in both populations were more protogynous and hence more outbred than later plants, which were more protandrous. These shed their pollen while silks are emerging and therefore have greater chance of inbreeding. The genetical consequences of this breeding system is evaluated in relation to heterosis.

4. The three-dimensional graph is compared with other graphic methods of representing flowering in maize. It is useful for comparing the uniformity and breeding systems of ordinary maize, inbreds and single-cross and double-cross populations.

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THE ANALYSIS OF MULTIPLE LINKAGE DATA

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In these notes I indicate methods applicable to the interpretation of multiple linkage tests involving three or more linked loci. The methods advocated are based on two extensions of Kosambi's simple and extremely useful theory of linkage relations, the purpose of the extensions being to widen the range of usefulness without losing the powerful simplicity of that theory.

In the first place Kosambi's theory (1) may be supplemented by the introduction of a quantity K whose value varies with position on the chromosome arm. I have shown elsewhere that we may expect high values of K to be realised for sets of loci in the neighbourhood of the centromere, and low values to occur near the termini of the chromosome, K taking the value unity at some point related to the middle of the arm. In a survey (2) of data on Chromosomes II and III of *Drosophila melanogaster* (not yet fully published), I found excellent agreement with these predictions. Thus it seems likely that in other organisms the value of K as derived from experiments on multiple crossing-over can be of some use in suggesting the location on the chromosome arm of the loci concerned. In Part I, I deal with the statistical problems involved in the estimation of K and of the trend of K in the cases of three and four linked loci, and discuss some actual data obtained for the Agouti-pallid region of Chromosome V in the mouse.

Part II is of more abstract interest and extends Kosambi's theory (without introducing K) to multiple recombination with as many as six linked loci. Here the mathematical method used is not identical with that of Kosambi but proceeds from the general theory of linkage relations developed by Sir Ronald Fisher and myself (2-7).

Part III introduces K into Kosambi's theory and gives an elementary way of generalising Kosambi's formulæ to the special but useful cases of several fairly closely linked loci such as are treated in I. 4.

I. ESTIMATION OF THE KOSAMBI COEFFICIENT

1. INTRODUCTION

The Kosambi coefficient, denoted by K, is defined (5) in terms of the recombination frequencies of 3 linked loci, A, B and D by means of the equations

$$K = \frac{y_1+y_2-y_{1+2}}{4y_1y_2y_{1+2}} = \frac{y_{12}}{2y_1y_2y_{1+2}}.$$

Here the suffixes 1 and 2 refer to the segments AB and BD, while the

suffix $1+2$ refers to the combined segment AD. Thus any three loci are characterised by a value of K. It will be seen that K is equal to $C/2y_{1+2}$, where C is the genetical coincidence for the two segments, defined by Stevens (8) as y_{12}/y_1y_2. Kosambi (1) obtained the addition formula

$$y_{1+2} = \frac{y_1+y_2}{1+4y_1y_2}$$

by an assumption equivalent to setting C equal to $2y_{1+2}$; *i.e.* to taking the value of K as unity. Regions of the chromosome in which K is not significantly different from unity have been called Kosambi regions (5), and in such regions interference is said to be at the Kosambi level (5). I have given (2, 5) theoretical reasons for supposing that in regions near the centromere K has a high value greater than unity, and diminishes steadily with increasing distance from the centromere, becoming relatively small near the terminus of an arm. Consequently there is a Kosambi region, where $K = 1$, situated medianly on the arm.

These expectations have been well fulfilled in the case of Chromosomes II and III in *Drosophila melanogaster* (2). Therefore, in the absence of evidence to the contrary, it seems reasonable in other organisms to use the observed K values as providing some indication of the distance of the loci concerned from the centromere, and of the orientation of segments relative to the centromere. This note is concerned with estimation of K values when data on 3 or 4 loci are available.

2. THE USE OF THREE POINT DATA

Backcross experiments provide the best determinations of the recombination fractions, and therefore of C and K. In such an experiment involving two segments AB and BD, suppose that out of a total of n organisms bred the observed class frequencies are

0	1	2	12
a_0	a_1	a_2	a_{12}

Here a_0 show recombination in neither 1 nor 2, a_1 show recombination in 1 but not in 2, a_2 show recombination in 2 but not in 1, while a_{12} show simultaneous recombination in 1 and 2. Thus

$n_1 = a_1+a_{12}$ show recombination in 1,
$n_2 = a_2+a_{12}$ show recombination in 2,
$n_{1+2} = a_1+a_2$ show recombination in the combined segment $1+2$ (AD),
$n_{12} = a_{12}$ show simultaneous recombination in 1 and 2.

The estimators of $y_1, y_2, y_{1+2}, y_{12}$ are

$$\hat{y}_1 = n_1/n, \hat{y}_2 = n_2/n, \hat{y}_{1+2} = n_{1+2}/n, \hat{y}_{12} = n_{12}/n.$$

The sampling variance of any such function is $V(y) = y(1-y)/n$.

The estimator of C is $\hat{C} = nn_{12}/n_1n_2$, and Stevens (*loc. cit.*) has shown that its sampling variance is

$$V(\hat{C}) = C(1-Cy_1-Cy_2-Cy_1y_2+2C^2y_1y_2)/ny_1y_2.$$

This may be estimated by inserting the estimates $\hat{y}_1, \ldots, \hat{C}$, and a crude test of the significance of the difference of C from unity (*i.e.* a test for the presence of genetical interference) may be based on the estimated standard error or on the variance. *E.g.* with the data

0	1	2	12	*n*
1116	182	375	24	1697

we have the percentage values

$$\hat{y}_1 = 12{\cdot}139, \quad \hat{y}_2 = 23{\cdot}512, \quad \hat{C} = 49{\cdot}551$$

Estimated $V(\hat{C}) = 84{\cdot}2176$ (per cent.)^2 $= 0{\cdot}008,421,76$

Estimated $s(\hat{C}) = 9{\cdot}1770$ per cent.

Thus $\hat{C} = 49{\cdot}55 \pm 9{\cdot}18$ per cent. which is significantly less than unity and indicative of genetical interference.

The maximum likelihood estimator of K is simply

$$\hat{K} = \hat{C}/2\hat{y}_{1+2} = n^2n_{12}/2n_1n_2n_{1+2}.$$

This statistic is biassed, the bias being given approximately by

$$\beta = \hat{K}\left(\frac{3}{n_{1+2}} - \frac{3}{n} - \frac{4\hat{K}n_{12}}{n^2}\right) \quad .$$

The estimated sampling variance is given by

$$V(K) = K^2\left\{\frac{1}{n_{12}} + \frac{5}{n_{1+2}} + \frac{2n_{12}}{n_1n_2} - \frac{1}{n_1} - \frac{1}{n_2} - \frac{4}{n} - \frac{4(n_1+n_2)\hat{K}}{n^2}\right\}$$

or may be got by inserting the estimates in

$$\frac{K^2}{n}\left\{\frac{1}{y_{12}} + \frac{5}{y_{1+2}} + 2C - \frac{1}{y_1} - \frac{1}{y_2} - 4 - 4K(y_1+y_2)\right\} \quad .$$

In the example we have

$$\hat{K} = 75{\cdot}48 \pm 15{\cdot}26 \text{ per cent.}, \qquad V(\hat{K}) = 232{\cdot}8934 \text{ (per cent.)}^2 \ .$$

The bias is not seriously large, since we have

$$\beta = 0{\cdot}004,041 = 0{\cdot}40 \text{ per cent.}$$

To test departure of K from unity we calculate

$$\chi^2_{[1]} = (\hat{K}-1)^2/V(K) = 4{\cdot}5317 \quad ,$$

where V(K) is now calculated from equation (1) with K put equal to unity.

K therefore deviates just significantly from unity.

In cases such as the present example when K is not very significantly different from unity a more refined procedure ought next to be used. We assume y_1, y_2 as free parameters and take y_{1+2}, y_{12} as given as functions of y_1, y_2 by Kosambi's formulæ. A maximum likelihood scoring technique gives amended estimates which are then used to calculate expected class frequencies for a $\chi^2_{(1)}$ fit to the observations. If the fit is good, *i.e.* if χ^2 is not significantly large then we may retain the hypothesis $K = 1$. If the fit is bad we may then estimate K as above, together with its estimated sampling variance and standard error.

3. THE USE OF FOUR POINT DATA

When data on four loci A, B, D, E are available, the appropriate treatment is determined by the closeness of the observed linkage between them. When the segments are sufficiently long for doubles to be at least moderately frequent, the best use of the data requires that K be calculated separately for the two triads ABD, BDE, the data being reduced to three point data in respect of each triad. If the values K_1, K_2 are significantly different then the direction of the trend may be taken as presumptive evidence of the orientation of the segment AE relative to the centromere O. *E.g.* if $K_1 > K_2$ the preferred order is OAE.

To test the significance of the difference $(K_1 - K_2)$ we may compare it with its sampling standard deviation using either a χ-, χ^2- or t-test. The variance of $(K_1 - K_2)$ is

$$V(K_1) - 2C(K_1, K_2) + V(K_2),$$

where $C(K_1, K_2)$ is the sampling covariance of K_1 and K_2, given approximately by

$$\hat{K}_1\hat{K}_2\left(\frac{n_{12}}{n_1n_2} + \frac{n_{23}}{n_2n_3} + \frac{n_{13}}{n_1n_3} + \frac{a_2}{n_2n_{1+2}} + \frac{a_2}{n_2n_{2+3}} + \frac{a_2}{n_{1+2}n_{2+3}} + \frac{n_{123}}{n_{12}n_{23}} - \frac{n_{123}}{n_1n_{23}} - \frac{n_{123}}{n_3n_{12}} - \frac{1}{n_2} - \frac{4}{n}\right)$$

This expression has been derived by means of a formula cognate to that given by Sir Ronald Fisher for the approximate sampling variance of a statistic (9). If $m_1, m_2, \ldots, m_r$ (totalling n) are the frequencies observed in r classes subject to a multinomial distribution, and if T_1, T_2 are homogeneous functions of degree 0 or 1 in $m_1, m_2, \ldots, m_r$ and n, then we have as the approximate sampling covariance of T_1 and T_2 the expression

$$C(T_1, T_2) = \sum_{i=1}^{r} m_i \frac{\partial T_1}{\partial m_i}\frac{\partial T_2}{\partial m_i} - n\frac{\partial T_1}{\partial n}\frac{\partial T_2}{\partial n}.$$

When $T_1 = T_2$, this becomes Fisher's formula

$$V(T) = \Sigma m\left(\frac{\partial T}{\partial m}\right)^2 - n\left(\frac{\partial T}{\partial n}\right)^2$$

Each formula holds exactly when T_1 and T_2 are linear functions of $m_1/n, \ldots\ldots, m_r/n$.

4. THE USE OF FOUR POINT DATA FOR SHORT SEGMENTS

When A and E are close together K_1 and K_2 will differ significantly. Double recombination classes will occur with small frequencies and triples will be absent. For if p, q, r are the three recombination fractions the expected frequencies of doubles and triples will be given to good approximation by the expressions given below (part III), namely :—

$$y_{12} = 2Kpq(p+q), \quad y_{23} = 2Kqr(q+r), \quad y_{13} = 2Kpr(p+2q+r),$$

$$y_{123} = 4K^3pqr\{(p+q)(q+r) - \tfrac{1}{3}q^2\}.$$

If the observations are

0	1	2	3	23	13	12	123	Total
a_0	a_1	a_2	a_3	a_{23}	a_{13}	a_{12}	0	n

then it may be shown by inspection of the maximum likelihood equations for the four unknowns p, q, r, K subject to joint estimation, that the estimators approximate closely to the " intuitive " ones

$\hat{p} = (a_1+a_{12}+a_{13})/n$, etc.

$\hat{K} = (a_{23}+a_{13}+a_{12})/D =$ (observed doubles)/(expected doubles).

Here D is the expected total frequency of doubles calculated as

$$D = n(y_{12}+y_{23}+y_{13})$$
$$= \tfrac{2}{3}(p+q+r)^3 - \tfrac{2}{3}(p^3+q^3+r^3)$$

estimated by inserting $\hat{p}$, $\hat{q}$, $\hat{r}$. Without serious error we may for this purpose take for $\hat{p}$, $\hat{q}$, $\hat{r}$ the quantities a_1/n, a_2/n, a_3/n. Thus we get the approximate estimator

$$\hat{K} = 3n^2(a_{23}+a_{13}+a_{12})/2(s_1^3 - s_3)$$

where $s_r = a_1^r + a_2^r + a_3^r$, $(r = 1, 2, 3, \ldots)$.

For the variance of K, we get by the approximate method of the last section

$$V(\hat{K}) = \hat{K}^2\left\{\frac{1}{a_{23}+a_{13}+a_{12}} + \frac{9(s_1^5 - 2s_1^2s_3 + s^5}{(s_1^3 - s_3)^2} - \frac{4}{n}\right\}$$

The first term is usually dominant so that to a rough approximation

$$V(\hat{K}) = \hat{K}^2/(\text{observed doubles}).$$

Example.—In a four point backcross experiment recently completed, of which a full account is not yet published, the four loci *Agouti*, *undulated*, *wellhaarig*, and *pallid* in the house mouse were employed. The following class frequencies were obtained.

	1	2	3	23	13	12	123	*n*
From female heterozygotes .	117	185	104	1	0	0	0	2503
From male heterozygotes .	115	111	56	1	0	1	0	2499
Total . . .	232	296	160	2	0	1	0	5002

On the combined data for males and females we have

$$\hat{p} = 4{\cdot}66 \text{ per cent.}, \quad \hat{q} = 5{\cdot}98 \text{ per cent.}, \quad \hat{r} = 3{\cdot}24 \text{ per cent.}$$

Observed doubles = 3. $s_1 = 688$, $s_1^2 = 47{\cdot}3344 \times 10^4$

$$s_3 = 42{\cdot}5175 \times 10^6, \qquad s_1^3 = 325{\cdot}6607 \times 10^6,$$

$$s_5 = 304{\cdot}9229 \times 10^{10}, \qquad s_1^5 = 1{\cdot}5415 \times 10^{14}$$

Thus $\hat{K} = 39{\cdot}76 \pm 23{\cdot}1105$ per cent., $V(\hat{K}) = 0{\cdot}055{,}263$, and $\hat{K}$ is significantly less than unity, its value being suggestive of a region towards the chromosome terminus or at least between mid-arm and the terminus. The segment *Agouti-pallid* is thus between 30-100 cM from the centromere according as to what is assumed for the map length of the arm.

The treatment is however rather inaccurate on account of the marked sex-difference of two of the recombination fractions, and is best carried out separately for the two sexes. We obtain

	K per cent.	Information
Females . . .	$16{\cdot}24 \pm 16{\cdot}41$	37·15
Males . . .	$96{\cdot}63 \pm 68{\cdot}29$	2·30
Combined . .	$20{\cdot}92 \pm 15{\cdot}09$	39·45

Thus in the females K is significantly low, but in the males does not differ significantly from 0 or unity. On the basis of the male and female data considered jointly we might legitimately assume the segment to be moderately distant from the centromere, since the difference between male and female K values is not itself significant.

5. SUMMARY

Methods of estimation are given for K and its sampling variance in the case of triads of loci, and for the covariance of K values in overlapping triads. It is shown how to estimate K in the case of three short segments with special reference to the *Agouti-pallid* region of Chromosome V in the house mouse. It is suggested that *Agouti* is moderately far from the centromere.

II. THE FREQUENCIES OF SIMULTANEOUS RECOMBINATION OF SEVERAL LINKED LOCI

1. INTRODUCTION

Kosambi (1) proposed the formula

$$y = \tfrac{1}{2}\tanh 2x$$

as an approximate representation of the relation between the genetical map-length x of the segment joining two loci and y the amount of genetical recombination between those loci. For 3 loci defining adjacent segments x_1 and x_2 this formula implies that

$$y_{12} = 2y_1y_2y_{1+2} = 2y_1y_2\frac{y_1+y_2}{1+4y_1y_2},$$

where y_{12} is the frequency of recombination in both simultaneously, and y_{1+2} is the frequency of recombination over the combined segment x_1+x_2. I have indicated (5, 7) the conditions under which these approximations are valid. If, in particular, the interference is represented by the function $4u\exp(-2u)$ employed in previous work (4-7), then, for a segment near the (metrical) mid-point of a long chromosome arm, we obtain the relation

$$y = \tfrac{1}{2}(1-e^{-2x}\cos 2x).$$

For segments less than 35 cM in map-length this function differs from $\frac{1}{2}\tanh 2x$ to an extent which in practice is quite inappreciable, the values of y given by the two formulæ being discrepant by at most 8 parts in 302. Thus Kosambi's formula receives a simple theoretical justification as an approximation likely to apply in segments median to long chromosome arms.

Using the methods indicated by previous work it has been shown (7) that there are simple expressions giving all recombination frequencies for 4 loci (3 segments) which also are likely to apply when all 4 loci are reasonably close to metrical mid arm. I gave the new relations

$$y_{13} = y_1y_3 - z_1z_3e^{-4x_2}, \qquad w_{12} = w_1w_2,$$

and

$$y_{123} = \mathrm{R}(w_1w_2w_3) + \tfrac{1}{2}z_1z_3(1-e^{-4x_2}).$$

Here

$$z = \tfrac{1}{2}e^{-2x}\sin 2x, \quad w = y+iz, \quad i = \sqrt{-1},$$

and

$$\mathrm{R}(w_1w_2w_3) = y_1y_2y_3 - y_1z_2z_3 - y_2z_1z_3 - y_3z_1z_2.$$

I showed further that these relations approximate to

$$y_{13} = y_1y_3\left\{1 - \frac{1-2y_2}{1+2y_2}\,\frac{(1-2y_1)(1-2y_3)}{1+4y_1y_3}\right\}$$

and

$$y_{123} = y_1y_{23} - y_2y_{13} + y_3y_{12}.$$

These equations may be regarded as natural generalisations of Kosambi's formulæ. While receiving their justification for short

segments only, like Kosambi's original formulæ they may often be found to apply in good approximation for larger segments. It will be seen that their form is consistent with the known disappearance of interference between long segments or widely separated segments.

It may be remarked in passing that the formulæ for 4 loci are adequate for the derivation of all formulæ required for 5 loci (4 segments) in virtue of identities of the type

$$y_{12,3+4} = y_{123} + y_{124} - 2y_{1234}.$$

In the present note I extend the results to give formulæ in y, z and x adequate to deal with as many as 6 loci (5 segments), and show how the formulæ may be adapted to apply to cases in which any or all of the pairs of segments are non-adjacent.

2. FORMULÆ

(a) *4 loci (3 adjacent segments).*—

$$y_{12} = y_1y_2 - z_1z_2, \quad y_{23} = y_2y_3 - z_2z_3,$$
$$y_{13} = y_1y_3 - z_1z_3e^{-4x_2},$$
$$y_{123} = y_1y_2y_3 - y_1z_2z_3 - y_2z_1z_3 - y_3z_1z_2 + \tfrac{1}{2}z_1z_3(1 - e^{-4x_2}).$$

(b) *5 loci (4 adjacent segments).*—Only one formula is essentially new, namely that for y_{1234}, all the others resulting either directly or by mere change of variables from those for 4 loci. However, it is instructive to write out the full set.

$$y_{12} = y_1y_2 - z_1z_2, \qquad y_{14} = y_1y_4 - z_1z_4e^{-4(x_2+x_3}$$
$$y_{23} = y_2y_3 - z_2z_3, \qquad y_{24} = y_2y_4 - z_2z_4e^{-4x_3}$$
$$y_{13} = y_1y_3 - z_1z_3e^{-4x_2}, \qquad y_{34} = y_3y_4 - z_3z_4,$$
$$y_{123} = y_1y_2y_3 - y_1z_2z_3 - y_2z_1z_3 - y_3z_1z_2 + \tfrac{1}{2}z_1z_3(1 - e^{-4x_2}),$$
$$y_{124} = y_1y_2y_4 - y_1z_2z_4e^{-2x_3} - y_1z_1z_4e^{-2x_3} - y_4z_1z_2 + \tfrac{1}{2}z_1z_4(1 - e^{-4x_2})e^{-4x_3},$$
$$y_{134} = y_1y_3y_4 - y_1z_3z_4 - y_3z_1z_4e^{-2x_3} - y_4z_1z_3e^{-2x_2} + \tfrac{1}{2}z_1z_4(1 - e^{-4x_2})e^{-4x_3},$$
$$y_{234} = y_2y_3y_4 - y_2z_3z_4 - y_3z_2z_4 - y_4z_2z_3 + \tfrac{1}{2}z_2z_4(1 - e^{-4x_2}),$$
$$\begin{aligned} y_{1234} = {} & y_1y_2y_3y_4 - y_1y_2z_3z_4 - y_2y_3z_1z_4 - y_1y_3z_2z_4 \\ & - y_1y_4z_2z_3 - y_2y_4z_1z_3 - y_2y_4z_1z_2 + z_1z_2z_3z_4 \\ & + \tfrac{1}{2}y_1z_2z_4(1 - e^{-4x_3}) + \tfrac{1}{2}y_2z_1z_4(1 - e^{-4x_3}) \\ & + \tfrac{1}{2}y_3z_1z_4(1 - e^{-4x_1}) + \tfrac{1}{2}y_4z_1z_3(1 - e^{-4x_2}) \\ & - \tfrac{1}{4}z_1z_4(1 - e^{-4x_2})(1 - e^{-4x_3}). \end{aligned}$$

(c) *6 loci (5 segments).*—Only two essentially new formulæ are required, namely :

$$\begin{aligned} y_{135} = {} & y_1y_3y_5 - y_1z_3z_5e^{-4x_4} - y_3z_1z_5e^{-4(x_2+x_4)} \\ & - y_5z_1z_3e^{-4x_2} + \tfrac{1}{2}z_1z_5(1 - e^{-4x_3})e^{-2(x_2+x_4)} \end{aligned}$$

$$
\begin{aligned}
y_{12345} = {} & y_1y_2y_3y_4y_5 - \Sigma z_1z_2y_3y_4y_5 \\
& -\tfrac{1}{2}\{z_1z_5y_3y_4(1-e^{-4x_2}) + z_1z_5y_2y_4(1-e^{-4x_3}) \\
& \quad + z_1z_5y_2y_3(1-e^{-4x_4}) + z_1z_3y_4y_5(1-e^{-4x_2}) \\
& \quad + z_1z_2y_4y_5(1-e^{-4x_3}) + z_1z_4y_3y_5(1-e^{-4x_2}) \\
& \quad + z_1z_4y_2y_5(1-e^{-4x_3}) + z_2z_5y_1y_3(1-e^{-4x_4}) \\
& \quad + z_2z_5y_1y_4(1-e^{-4x_3}) + z_3z_5y_1y_2(1-e^{-4x_4})\} \\
& -\tfrac{1}{2}\{z_1z_3z_4z_5(1-e^{-2x_2}) + z_1z_2z_4z_5(1-e^{-2x_3}) \\
& \quad + z_1z_2z_3z_5(1-e^{-2x_4})\} \\
& +\tfrac{1}{4}(z_1z_5y_2 + z_2z_5y_1)(1-e^{-4x_3})(1-e^{-4x_4}) \\
& +\tfrac{1}{4}(z_1z_5y_3 + z_3z_5y_1)(1-e^{-4x_2})(1-e^{-4x_4}) \\
& +\tfrac{1}{4}(z_1z_5y_4 + z_4z_5y_1)(1-e^{-4x_2})(1-e^{-4x_3}) \\
& -\tfrac{1}{8}z_1z_5(1-e^{-4x_2})(1-e^{-4x_3})(1-e^{-4x_4}).
\end{aligned}
$$

(d) *Extension of formulæ to separated segments.*—It is easy to generalise any formula relating to contiguous segments so that it applies to non-adjacent ones. For example, the expression for y_{1357} relating to intervals x_1, x_3, x_5, x_7 out of the contiguous set x_1, x_2, x_3, x_4, x_5, x_6, x_7, can be derived as follows :

If x_2, x_4, x_6 were all zero so that 1, 3, 5 and 7 were contiguous we would have

$$
\begin{aligned}
y_{1357} = {} & y_1y_3y_5y_7 - z_1z_3y_5y_7 - z_1z_5y_3y_7 - z_1z_7y_3y_5 \\
& - z_3z_5y_1y_7 - z_3z_7y_1y_5 - z_5z_7y_1y_3 \\
& + z_1z_3z_5z_7 + \tfrac{1}{2}z_1z_7y_5(1-e^{-4x_2}) + \tfrac{1}{2}z_1z_7y_3(1-e^{-4x_4}) \\
& + \tfrac{1}{2}z_1z_5y_7(1-e^{-4x_2}) + \tfrac{1}{2}z_3z_7y_1(1-e^{-4x_4}) \\
& - \tfrac{1}{4}z_1z_7(1-e^{-4x_2})(1-e^{-4x_4}).
\end{aligned}
$$

When x_2, x_4, x_6 are not zero this formula becomes

$$
\begin{aligned}
y_{1357} = {} & y_1y_3y_5y_7 - z_1z_3y_5y_7e^{-4x_2} - z_1z_5y_3y_7e^{-4(x_2+x_4)} \\
& - z_1z_7y_3y_5e^{-4(x_2+x_4+x_6)} - z_3z_5y_1y_7e^{-4x_4} \\
& - z_3z_7y_1y_5e^{-4(x_4+x_6)} - z_5z_7y_1y_3e^{-4x_6} \\
& + z_1z_3z_5z_7e^{-4(x_2+x_6)} \\
& + \tfrac{1}{2}z_1z_7y_5(1-e^{-4x_2})e^{-4(x_2+x_4+x_6)} + \tfrac{1}{2}z_1z_7y_3(1-e^{-4x_4})e^{-4(x_2+x_4+x_6)} \\
& + \tfrac{1}{2}z_1z_5y_7(1-e^{-4x_2})e^{-4(x_2+x_4)} + \tfrac{1}{2}z_3z_7y_1(1-e^{-4x_4})e^{-4(x_4+x_6)} \\
& - \tfrac{1}{4}z_1z_7(1-e^{-4x_2})(1-e^{-4x_4})e^{-4(x_2+x_4+x_6)}
\end{aligned}
$$

The rule of derivation consists merely of inserting an attenuation factor corresponding to each term $z_j\ z_k$ as written and detemined by the total length of " gap intervals " between the segments j and k.

3. METHOD OF PROOF OF THE FORMULÆ

For $(k+1)$ loci (k contiguous segments $x_1, x_2, \ldots, x_k$) infinitely distant both from the centromere and the terminus of the chromosome arm we have a generating function (5) which takes the value

$$P(\lambda_1, \lambda_2 \ldots, \lambda_k) = \tfrac{1}{2}Q \exp(-\sum_{j=1}^{k} D_j),$$

where $$D_j = 2x_j \quad (j = 1, 2, \ldots, k),$$

and $$Q = 2C_1C_2 \ldots C_k + \Sigma S_1C_2 \ldots C_kL_1 + \Sigma S_1S_2C_3 \ldots C_kL_{12} + \ldots \ldots + S_1S_2 \ldots S_kL_{12\ldots k}$$

with $$C_j = \cosh 2\sqrt{\lambda_j}, \qquad S_j = \sinh 2\sqrt{\lambda_j}.$$

In a typical item such as $S_1S_2C_3S_4C_5C_6S_7C_8L_{1247}$ the L symbol L_{1247} is to be read as the function

$$\left(\frac{\sqrt{\lambda_1}\sqrt{\lambda_4}}{\sqrt{\lambda_2}\sqrt{\lambda_7}} + \frac{\sqrt{\lambda_2}\sqrt{\lambda_7}}{\sqrt{\lambda_1}\sqrt{\lambda_4}}\right).$$

Similarly L_{34568} in $C_1C_2S_3S_4S_5S_6C_7S_8C_9C_{10}L_{34568}$ is

$$\left(\frac{\sqrt{\lambda_3}\sqrt{\lambda_5}\sqrt{\lambda_8}}{\sqrt{\lambda_4}\sqrt{\lambda_6}} + \frac{\sqrt{\lambda_4}\sqrt{\lambda_6}}{\sqrt{\lambda_3}\sqrt{\lambda_5}\sqrt{\lambda_8}}\right).$$

The multiple segmental recombination fractions can be calculated from the 2^n quantities obtained from P by substituting values $+1$ or -1 for the λ's (5). For example, if we set

$$\lambda_{n_1} = \lambda_{n_2} = \ldots = \lambda_{n_r} = -1$$

and all other λ's equal to $+1$, the resulting value of P is equal to

$$P_{n_1n_2 \ldots n_r} = 1 - 2y_{n_1+n_2+\ldots n_r}$$

where $y_{n_1+n_2+\ldots+n_r}$ is the probability that the sum of the numbers of exchange points in the segments $x_{n_1}, x_{n_2}, \ldots, x_{n_r}$ will be odd.

It is easy to verify the elementary identity

$$1 - 2y_{n_1+n_2+\ldots+n_r} = 1 - 2\Sigma y_{n_1} + 4\Sigma y_{n_1n_2} - \ldots + (-)^r 2^r y_{n_1n_2 \ldots n_r},$$

where $y_{n_1n_2}$ is the probability that segments x_{n_1} and x_{n_2} will show simultaneous recombination, and so on. Thus the segmental recombination fractions $y_{n_1n_2}, y_{n_1n_2n_3}, \ldots, y_{12\ldots k}$ can be calculated inductively.

For example, with 3 adjacent segments we have

$$\begin{aligned} 1 - 2(y_1+y_3) + 4y_{13} &= 1 - 2y_{1+3} = P_{13} = P(-1, 1, -1) \\ &= e^{-(D_1+D_3)}(\cos D_1 \cos D_3 - e^{-2D_2} \sin D_1 \sin D_3) \\ &= (1-2y_1)(1-2y_3) - 4z_1z_3e^{-4x_2}. \end{aligned}$$

Thus $$y_{13} = y_1y_3 - z_1z_3e^{-4x_2}.$$

Similarly

$$1-2(y_1+y_2+y_3)+4(y_{12}+y_{23}+y_{13})-8y_{123}$$
$$= 1-2y_{1+2+3} = P_{123} = P(-1, -1, -1)$$
$$= e^{-(D_1+D_2+D_3)}(\cos D_1 \cos D_2 \cos D_3 - \sin D_1 \sin D_2 \cos D_3$$
$$-e^{-2D_3} \sin D_1 \cos D_2 \sin D_3 - \cos D_1, \sin D_2 \sin D_3)$$

which ultimately gives the value of y_{123}.

4. FORMULÆ IN TERMS OF RECOMBINATION FRACTIONS ONLY

The substitutions

$$e^{-4x_r} \doteq (1-2y_r)/(1+2y_r)$$
$$z_r z_s \doteq (1-2y_r)(1-2y_s)/(1+4y_r y_s)$$

give formulæ generating those of Kosambi (1) and Owen (7). With $(k+1)$ linked loci they could be used for fitting the k parameters $y_1, y_2, \ldots, y_k$ to observed recombination data by maximum likelihood scoring.

5. THE AUXILIARY QUANTITY z

$$z = \tfrac{1}{2}e^{-2x} \sin 2x$$

is the imaginary part of w where

$$1-2w = e^{-2x(1+i)},$$

so that $w = y+iz$ satisfies Trow's formula for adjacent segments at mid-arm.

i.e. $$w_{1+2} = w_1+w_2-2w_1w_2$$

Thus the effect of interference is formally removed for adjacent segments by the use of w, which may be called the *complex recombination fraction* or " *complexit* ", its components y and z being named respectively the " *recombit* " and the " *cocombit* ".

For short segments we have the expansions

$$y = \tfrac{1}{2}(1-e^{-2x}\cos 2x) = x-\tfrac{4}{3}x^3+\ldots$$

as in Kosambi's formula.

$$z = \tfrac{1}{2}e^{-2x} \sin 2x = x-2x^2+\tfrac{4}{3}x^3+\ldots$$
$$x = y+\tfrac{4}{3}y^3+\ldots$$

Therefore
$$z = y-2y^2+\tfrac{8}{3}y^3+\ldots$$
$$= y(1-2y)/(1-\tfrac{8}{3}y^2)+o(y^4).$$

The second order approximation to the cocombit is therefore $y(1-2y)$, but its use is rather less effective than that of the approximation for $z_r z_s$. It may be noted, however, that a good second order approxima-

tion is provided by $y(1-1{\cdot}56y)$. The following table gives percentage values of x (cM), of y and of $\frac{1}{2}\tanh 2x$ and also of z and of $y(1-1{\cdot}56y)$.

x cM	$\frac{1}{2}\tanh 2x$	y	z	$y(1-1{\cdot}56y)$
0	0	0	0	0
5	4·984	4·984	4·517	4·597
10	9·869	9·879	8·133	8·357
15	14·566	14·614	10·946	11·282
20	18·998	19·130	13·052	13·421
25	23·106	23·386	14·540	14·854
30	26·852	27·352	15·494	15·681
35	30·218	31·010	15·995	16·009
40	33·202	34·348	16·116	15·943
45	35·815	37·364	15·924	15·585
50	38·080	40·062	15·478	15·025
55	40·025	42·451	14·833	14·339
60	41·683	44·543	14·036	13·591
65	43·086	46·355	13·130	12·834
70	44·268	47·904	12·150	12·105
75	45·257	49·211	11·129	11·432
80	46·084	50·029	10·091	10·983
85	46·770	51·175	9·058	10·320
90	47·340	51·894	8·049	9·883
95	47·812	52·462	7·077	9·527
100	48·201	52·904	6·153	9·242

6. LOCI PROXIMAL TO CENTROMERE ON A LONG ARM

It is easy to derive results applying to segments on a long arm which are at finite distances from the centromere. We replace the map distance x, wherever it appears in the existing formulæ, by the interference metric t and treat the centromere as having the properties of a localised exchange point (4, 5).

Consider 3 adjacent segments 0, 1, 2 which have the metrical specification $(0, t_0)$, (t_0, t_1), (t_1, t_2). We have

$$y_{02} = y_0\eta_2 - z_0\zeta_2 e^{-4D_1}$$

where

$$\eta = \tfrac{1}{2}(1-e^{-2x}\cos 2D),\ \zeta = \tfrac{1}{2}e^{-2D}\sin 2D$$

and D is now metrical length, *i.e.* $D_1 = (t_1 - t_0)$.

The probability of recombination in 2 conditional on recombination in 0 is

$$y_{02}/y_0 = \eta_2 - e^{-4D_1}\zeta_2(z_0/y_0).$$

The limit of this probability as $t_0 \to 0$ is clearly the probability of recombination in 2 conditional on there being an exchange point at $t = 0$, and therefore gives the recombination over a segment (t_1, t_2) at metrical distance t_1 from the centromere as

$$y_2 = \eta_2 - e^{-4t_1}\zeta_2$$

as given by Owen (4).

Similarly for simultaneous recombination in separated intervals we have

$$y_{13} = \eta_1\eta_3 - \zeta_1\zeta_3 e^{-4D_2} - e^{-4t_1}\{\zeta_1\eta_3 + \zeta_3\eta_1 e^{-4D_2} - \tfrac{1}{2}\zeta_3(1 - e^{-4D_1})e^{-4D_2}\}.$$

Also

$$\begin{aligned} y_{123} = \eta_1\eta_2\eta_3 &- (\eta_1\zeta_2\zeta_3 + \eta_2\zeta_1\zeta_3 + \eta_3\zeta_1\zeta_2) + \tfrac{1}{2}\zeta_1\zeta_3(1 - e^{-4D_2}) \\ &- e^{-4t_1}\{\eta_1\eta_2\zeta_3 + \eta_2\eta_3\zeta_1 + \eta_1\eta_3\zeta_2 - \zeta_1\zeta_2\zeta_3 \\ &\quad - \tfrac{1}{2}\eta_1\zeta_3(1 - e^{-4D_2}) - \tfrac{1}{2}\eta_2\zeta_3(1 - e^{-4D_1}) \\ &\quad - \tfrac{1}{2}\zeta_2\eta_3(1 - e^{-4D_1}) - \tfrac{1}{4}\zeta_3(1 - e^{-4D_1})(1 - e^{-4D_2})\}. \end{aligned}$$

7. SUMMARY

Generalisations of Kosambi's formulæ are obtained serving to give the frequencies of multiple recombination in as many as 5 adjacent or separated segments. The formulæ are expressed in terms of recombination fractions for single segments and auxiliary quantities z. It is shown how to obtain complete generalisations of Kosambi's formulæ, expressed purely in terms of the recombination fractions.

III. ELEMENTARY FORMULÆ FOR MULTIPLE RECOMBINATION OF CLOSELY LINKED LOCI

1. INTRODUCTION

It has been seen that for contiguous segments 1 and 2 Kosambi (1) gives the useful addition formula

$$y_{1+2} = (y_1 + y_2)/(1 + 4y_1y_2)$$

connecting the recombination fractions y_1, y_2 and y_{1+2} in the segments 1 and 2 and the joint segment 1+2. This has been found to be substantially correct for long segments in the mouse (10), in rice (11), and in the sweet pea (12) and to hold for loci situated medianly in the arms of chromosomes VI and III in *Drosophila melanogaster* (2). Theoretical reasons have been advanced why this should be so (5, 7).

Since the frequency of simultaneous recombination is given always by

$$y_{12} = \tfrac{1}{2}(y_1 + y_2 - y_{1+2})$$

it follows from Kosambi's formula that the frequency of doubles is given by

$$y_{12} = 2y_1y_2(y_1 + y_2)/(1 + 4y_1y_2)$$

It was shown above that for non-contiguous segments an appropriate generalisation of this result is

$$y_{13} = y_1y_3\left\{1 - \frac{1 - 2y_2}{1 + 2y_2}\,\frac{(1 - 2y_1)(1 - 2y_3)}{1 + 4y_1y_3}\right\}.$$

For short segments when y_1, y_2, y_3 are small, if we retain only terms of the third order we get the formulæ

$$y_{12} = 2y_1y_2(y_1+y_2), \quad y_{13} = 2y_1y_3(y_1+2y_2+y_3).$$

Similar formulæ could be got for the frequencies of triple, quadruple crossing over, etc. using the previous results. However, Professor Sir Ronald Fisher suggested to me that they could be derived rather simply by a completely elementary method, which may be illustrated by the case of 4 linked loci (3 contiguous segments).

We note that if segment 3 is so short that y_3 may be replaced by the differential dy then the probability of simultaneous recombination in segment 1 and the segment dy is

$$y_{13} = 2y_1(y_1+2y_2)dy \quad . \quad . \quad . \quad (1)$$

Equation (1) also implies that the probability of recombination in 1 conditional on recombination in dy is

$$y_{13}/dy = 2y_1(y_1+2y_2) \quad . \quad . \quad . \quad (2)$$

Results (1) and (2) enable the frequencies of simultaneous recombination in any number of short contiguous segments to be calculated inductively provided that a further simple assumption is made. This assumption is in accordance with recombination theory as previously developed. We suppose that a cross-over point once established effectively divides the chromosome into non-interfering regions.

Let now p, q, r be the frequencies of recombination in contiguous segments AB, BC, CD supposed short but not infinitesimal. Consider an elementary segment dy located at a point P interior to BC. Then to the present approximation the recombination over BP is y and over PC is $(q-y)$. By (1) the probability of simultaneous recombination in AB and dy is

$$2p(p+2y)dy.$$

But by (2) the probability of recombination in CD conditional on recombination in dy is

$$2r(r+2\overline{q-y}).$$

Thus the probability of recombination simultaneously in AB, dy and CD is

$$4pr(p+2y)(r+2q-2y)dy.$$

It is now easy to see that, on account of the assumed smallness of p, q and r, the probability of simultaneous recombination y_{12} in AB, BC and CD is merely

$$\int_0^q 4pr(p+2y)(r+2q-2y)dy$$

which is

$$4pr\{(p+q)(q+r)-\tfrac{1}{3}q^2\}.$$

The results of this method are assembled for 4, 5 and 6 loci in section 3. It will be seen that a parameter K has been introduced. This will be discussed below.

2. THE KOSAMBI COEFFICIENT

The Kosambi coefficient K for three linked loci is defined as

$$K = \frac{y_1+y_2-y_{1+2}}{4y_1y_2y_{1+2}} = \frac{y_{12}}{2y_1y_2y_{1+2}}.$$

The value of K is in general different from unity. K is equal to unity, if and only if, Kosambi's formula holds exactly. The value of K as estimated from 3 point data may be used to test agreement with Kosambi's formula. When K is significantly less than unity then the genetical interference which is operating must be concluded to be more intense than that predicted by Kosambi's formula. The opposite conclusion is indicated by an observed K significantly exceeding unity. Reasons have been given (6) for supposing that in regions near the centromere K is large, and thereafter diminishes steadily becoming less than unity in regions near the terminus of a chromosome arm. At some intermediate region (actually near metrical mid-arm) K is approximately unity and Kosambi's formula will apply there. These expectations are confirmed by the data on Chromosomes II and III of *Drosophila melanogaster* (2). Thus an observed value of K is some indication of the location of the triplet of loci on the chromosome arm.

Inclusion of the parameter K modifies the formulæ for short segments in a simple way and confers generality upon them.

If K is defined as above then we have identically for the frequency of doubles

$$y_{12} = 2Ky_1y_2y_{1+2} = 2Ky_1y_2(y_1+y_2)/(1+4Ky_1y_2),$$

which for short segments is effectively

$$y_{12} = 2Ky_1y_2(y_1+y_2) = 2Ky_1(y_1y_2+y_2^2)$$

Now suppose that the segment y_2 is increased to be y_2+dy by the addition of an elementary segment dy. Then the resulting increase in y_{12} is given by

$$dy_{12} = 2Ky_1(y_1+2y_2)dy+2y_1y_2(y_1+y_2)\frac{\partial K}{\partial y_2}dy$$

The second term is of the third degree in y_1, y_2 and consequently may be neglected in the present approximation. Thus

$$dy_{12} = 2Ky_1(y_1+2y_2)dy$$

and the probability of simultaneous recombination in AB and dy becomes

$$2Kp(p+2y)dy,$$

K appearing merely as a multiplier.

To the present order of accuracy therefore, K is to be regarded as a constant over the total region occupied by the segments and the formulæ are merely multiplied by powers of K.

3. FORMULÆ

(a) *Notation.*—p, q, r, s, t, denote contiguous segments taken in order. It is convenient to define the following symbols.

$$(p, q, r) = (p+q)(q+r) - \tfrac{1}{3}q^2$$
$$(p, q, r, s) = (p+q)(q+r)(r+s) - \tfrac{1}{3}q^2(r+s) - \tfrac{1}{3}r^2(p+q)$$
$$(p, q, r, s, t) = (p, q, r)(r, s, t) - \tfrac{1}{3}r^2(p+q)(r+t)$$

(b) *4 loci (3 segments).*—

$$y_{12} = 2\mathrm{K}pq(p+q), \qquad y_{23} = 2\mathrm{K}qr(q+r)$$
$$y_{13} = 2\mathrm{K}pr(p+2q+r)$$
$$y_{123} = 4\mathrm{K}^2pqr(p, q, r)$$

(c) *5 loci (4 segments).*—

$$y_{12} = 2\mathrm{K}pq(p+q), \qquad y_{13} = 2\mathrm{K}pr(p+2q+r)$$
$$y_{14} = 2\mathrm{K}ps(p+2q+r+s), \qquad y_{23} = 2\mathrm{K}qr(q+r),$$
$$y_{24} = 2\mathrm{K}qs(q+s), \qquad y_{34} = 2\mathrm{K}rs(r+s),$$
$$y_{234} = 4\mathrm{K}^2qrs(q, r, s), \qquad y_{123} = 4\mathrm{K}^2pqr(p, q, r),$$
$$y_{124} = 4\mathrm{K}^2pqs\{(p+q)(q+2r+s) - \tfrac{1}{3}q^2\},$$
$$y_{134} = 4\mathrm{K}^2prs\{(r+s)(p+2q+r) - \tfrac{1}{3}r^2\},$$
$$y_{1234} = 8\mathrm{K}^3pqrs(p, q, r, s).$$

It will be seen that with the exception of the last three, these expressions result *mutatis mutandi* from the appropriate 3-point formulæ. For instance y_{14} is derived from $2\mathrm{K}pr(p+2q+r)$ by the substitutions s for r, $q+r$ for q ($q+r$ being to the present degree of approximation the recombination in the separating segment $(2+3)$). The formulæ for y_{124} and y_{134} contain nothing essentially new being calculated from the relations

$$y_{124} = y_{123+4} - y_{123}, \qquad y_{134} = y_{1+234} - y_{234},$$

which hold in the present approximation. Their form shows them however to be derivable by a simple rule. We may write

$$y_{124} = 4\mathrm{K}^2pqs(p, q, s)^1$$

where $(p, q, s)^1$ results from (p, q, s) by inserting in the item $(q+s)$ the term $2r$ corresponding to the separating segment r.

(d) *6 loci (5 segments).*—Formulæ for two segments such as

$$y_{15} = 2\mathrm{K}pt(p+2q+2r+2s+t)$$

follow trivially from the previous results.

The formulæ for three or four segments follow either directly or by application of the rule for separating segments, *e.g.*

$$y_{125} = 4K^2pqt(p, q, t)^1 = 4K^2pqt\{(p+q)(q+2r+2s+t) - \tfrac{1}{3}q^2\},$$
$$y_{135} = 4K^2prt(p, r, t)^1 = 4K^2prt\{(p+2q+r)(r+2s+t) - \tfrac{1}{3}r^2\},$$
$$y_{1235} = 8K^3pqrt(p, q, r, t)^1$$
$$= 8K^3pqrt\{(p+q)(q+r)(r+2s+t) - \tfrac{1}{3}q^2(r+2s+t) - \tfrac{1}{3}r^2(p+q)\},$$
$$y_{1245} = 8K^3pqst(p, q, s, t)^1$$
$$= 8K^3pqst\{(p+q)(q+2r+s)(s+t) - \tfrac{1}{3}q^2(s+t) - \tfrac{1}{3}s^2(p+q)\}.$$

The formula for 5 segments is essentially new and can be found only by integration.

$$y_{12345} = 16K^4pqrst(p, q, r, s, t)$$

4. VERIFICATION

The expression

$$y_{123} = y_1y_2y_3 - y_1z_2z_3 - y_2z_1z_3 - y_3z_1z_2 + \tfrac{1}{2}z_1z_3(1 - e^{-4x_1}),$$

given previously (loc. cit.) reduces to the form $4pqr(p, q, r)$ on putting

$$z = y - 2y^2 + \tfrac{8}{3}y^3,$$
$$e^{-4x} = 1 - 4y + 8y^2 - 16y^3 + \ldots = (1-2y)(1+2y)$$
$$= \exp\{-4(y + \tfrac{8}{3}y^3)\} + o(y^4),$$

and retaining only terms of the fifth degree. This serves as a mutual check of both formulæ.

A similar comparison may be made with the generalised Kosambi formula given by the writer (loc cit.) namely

$$y_{123} = py_{23} - qy_{13} + ry_{12}$$

with y_{12}, y_{13} given by Kosambi's formula and

$$y_{13} = pr\left\{1 - \frac{1-2q}{1+2q}\,\frac{(1-2p)(1-2r)}{1+4pr}\right\}.$$

With small segments we have correct to the 5th degree

$$y_{123} = 4pqr.2q(p+q+r).$$

Consequently the generalised Kosambi formula implies rather a higher frequency of triples (about 50 per cent. more). This is because it is essentially a quadratic approximation and not a cubic one.

5. SUMMARY

An elementary method is developed by which the frequencies of simultaneous recombination of several closely linked loci can be specified, and formulæ are given for as many as 6 such loci.

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LINKAGE IN HEXASOMIC INHERITANCE

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1. INTRODUCTION

In studies on genetic linkage, the primary object is to form estimates of the frequencies of the modes of gamete formation from data on the gametic output of individuals of known genotype. With disomic inheritance one parameter, the recombination fraction, enters into the law of inheritance for pairs of linked loci and in the absence of differential viability, this can be estimated from the gametic output of either of the two types of double heterozygote. With polysomic inheritance, a multiplicity of segregating genotypes and of modes of gamete formation makes the analysis more complex.

2. TETRASOMIC INHERITANCE AT TWO LINKED LOCI

With two linked loci and tetrasomic inheritance, there are eleven modes of gamete formation. An example of this genetic situation has been discovered in *Lythrum salicaria* (Fisher, 1949). Fisher (1947) has considered this case in some detail. He has shown how, in general, the heterogenic genotypes can be classified into isomorphic sets such that all members of a set can be generated from any constituent member by systematic gene substitutions. It follows that genotypes which belong to the same set yield populations of gametes with equivalent frequencies. In accordance with a generalised form of Mendel's law, there is determined for any genotype, a gametic series for each mode of gamete formation. The gametic output is then expressible in the form of a matrix with the rows corresponding with the different modes of gamete formation and the columns with the different gametic genotypes. In most cases, the rank of the gametic matrix is less than the number of modes of gamete formation so that the gametic output from a particular isomorphic set of genotypes is deficient in some of the information sought. A detailed analysis is required in order to find out what information becomes available on considering genotypes from different isomorphic sets. The results of the analysis for two linked tetrasomic loci are summarised in fig. 1, which is reproduced from a wall diagram in the Department of Genetics, Cambridge, by the kind permission of Sir Ronald Fisher.

With only two alleles at each of the two loci, the frequencies of all eleven modes of gamete formation cannot be estimated. However,

six can be estimated as well as four independent linear combinations involving the other five.

Fisher's graphical analysis of the 24 gametic matrices in tetrasomic inheritance with loci

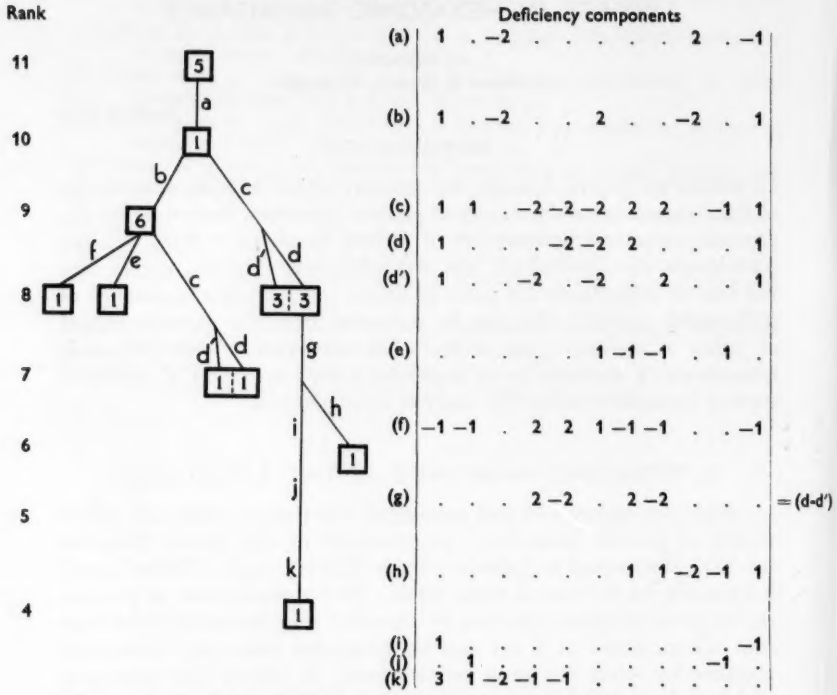

FIG. 1.—Numbers of isomorphic sets having gametic matrices of different rank, with the particular components of deficiency in which they differ.

3. HEXASOMIC INHERITANCE AT TWO LINKED LOCI

It is of some interest to consider the problem presented by linkage between two loci showing hexasomic inheritance. Although hexasomic inheritance has not yet been demonstrated in any organism, it is not unexpected in autohexaploids.

It might be noted here that polyploids are conveniently classified both according to the origin of their germ plasm and according to their breeding behaviour. If polyploidy has originated from a cross involving only one species, the new individual is an auto-polyploid whereas if more than one species was involved in the cross, it is an allo-polyploid. Polyploids which on breeding always behave as diploids are properly called amphi-diploids. These are to be distinguished from polyploids which exhibit polysomic inheritance and in which, at meiosis, a gene has more than one possible partner.

Genetical behaviour might not be of the same nature throughout the whole germ plasm and it is conceivable that inheritance might be polysomic in one set of chromosomes and disomic in another.

With two linked loci and hexasomic inheritance, there are forty modes of gamete formation. Using Fisher's enumeration (1947, p. 72) these are shown in table 1 where, for each mode of formation,

TABLE 1

Mode of formation	Typical gamete	Mode of formation	Typical gamete
1	$a_1b_1/a_2b_2/a_3b_3$	21	$a_1b_1/a_2b_2/a_2b_3$
2	$a_1b_1/a_1b_1/a_2b_2$	22	$a_1b_1/a_2b_2/a_3b_2$
3	$a_1b_1/a_2b_2/a_3b_4$	23	$a_1b_1/a_2b_2/a_2b_4$
4	$a_1b_1/a_2b_1/a_3b_2$	24	$a_1b_1/a_2b_2/a_4b_2$
5	$a_1b_1/a_1b_1/a_2b_3$	25	$a_1b_1/a_1b_2/a_3b_4$
6	$a_1b_1/a_2b_2/a_4b_5$	26	$a_1b_1/a_2b_1/a_4b_2$
7	$a_1b_1/a_2b_1/a_3b_3$	27	$a_1b_1/a_1b_2/a_3b_2$
8	$a_1b_1/a_2b_2/a_2b_2$	28	$a_1b_1/a_2b_1/a_2b_2$
9	$a_1b_1/a_2b_2/a_3b_4$	29	$a_1b_1/a_1b_2/a_2b_2$
10	$a_1b_2/a_2b_2/a_3b_3$	30	$a_1b_1/a_2b_1/a_2b_2$
11	$a_1b_1/a_1b_2/a_2b_1$	31	$a_1b_2/a_1b_2/a_4b_5$
12	$a_1b_2/a_2b_4/a_3b_4$	32	$a_2b_1/a_2b_1/a_3b_4$
13	$a_1b_2/a_1b_2/a_3b_4$	33	$a_1b_2/a_1b_2/a_3b_4$
14	$a_1b_2/a_2b_1/a_3b_4$	34	$a_2b_1/a_2b_1/a_4b_2$
15	$a_1b_2/a_2b_2/a_3b_4$	35	$a_1b_2/a_1b_2/a_4b_1$
16	$a_1b_2/a_2b_1/a_3b_2$	36	$a_2b_1/a_2b_1/a_1b_4$
17	$a_1b_2/a_2b_1/a_2b_1$	37	$a_1b_2/a_1b_2/a_2b_1$
18	$a_1b_2/a_2b_2/a_4b_5$	38	$a_2b_1/a_2b_1/a_1b_2$
19	$a_1b_2/a_2b_2/a_2b_4$	39	$a_1b_2/a_1b_2/a_2b_2$
20	$a_1b_2/a_2b_2/a_2b_1$	40	$a_2b_1/a_2b_1/a_2b_2$

there is given the genotype of a typical gamete that would arise from the parental genotype $a_1b_1/a_2b_2/a_3b_3/a_4b_4/a_5b_5/a_6b_6$.

There are sixty hexasomic genotypes with two alleles at each of

TABLE 2

Numbers of isomorphic sets of digenic hexasomic genotypes classified according to the partitions at two loci

Partitions	(51)	(42)	(3^2)
(51)	2	2	1
(42)	2	3	2
(3^2)	1	2	2

two linked loci and these fall into seventeen isomorphic sets of which seven are self-conjugate, the remainder comprising five conjugate pairs. These sets can be classified as shown in table 2 according to the partitions at the two loci.

The number of gametic genotypes to which any parental genotype

can give rise is determined by the partitions at both loci. These numbers are shown in table 3.

TABLE 3

Numbers of gametic genotypes to which any parental genotype can lead

Partitions	(51)	(42)	(3^2)
(51)	13	16	16
(42)	16	20	20
(3^2)	16	20	20

4. THE GAMETIC MATRICES

Since parental genotypes belonging to the same isomorphic set have equivalent gametic matrices and as each of the gametic matrices for two conjugate isomorphic sets can readily be developed from the other, it is sufficient if we construct gametic matrices for one parental genotype from each of the seven self-conjugate sets and from one of each pair of conjugate sets (see tables 5-16).

As the rank of any one of these gametic matrices cannot exceed two less than the number of gametic genotypes which enter into it, no parental genotype used alone can lead to estimates of more than eighteen independent linear combinations of the forty frequencies. The actual values of the ranks of the twelve gametic matrices are shown, with typical parental genotypes, in table 4.

TABLE 4

Ranks of gametic matrices

	(51)	(42)	(3^2)
(51)	*AB*|*ab*|*ab*|*ab*|*ab* 11 *Ab*|*aB*|*ab*|*ab*|*ab* 11	*Ab*|*Ab*|*aB*|*ab*|*ab*|*ab* : 13 *AB*|*Ab*|*ab*|*ab*|*ab* : 13	*AB*|*Ab*|*Ab*|*ab*|*ab*|*ab* : 11
(42)	...	*AB*|*AB*|*ab*|*ab*|*ab*|*ab* : 16 *AB*|*Ab*|*aB*|*ab*|*ab*|*ab* : 16 *Ab*|*Ab*|*aB*|*aB*|*ab*|*ab* : 16	*AB*|*AB*|*Ab*|*ab*|*ab*|*ab* : 14 *AB*|*Ab*|*Ab*|*aB*|*ab*|*ab* : 6
(3^2)	...	...	*AB*|*AB*|*AB*|*ab*|*ab*|*ab* : 10 *AB*|*AB*|*Ab*|*aB*|*ab*|*ab* : 10

Beneath each gametic matrix there is indicated a series of gametes whose classification would lead to the maximum number of independent linear combinations available for estimation from any parental genotype which belongs to the given isomorphic set. The number of gametes in any such series is, of course, equal to the rank of the gametic matrix. To take an example, the self-conjugate set of four genotypes which includes bisimplex coupling $(AB)(ab)_5$, can yield estimates of eleven independent linear combinations and at the bottom of table 5 there is indicated a series of eleven columns. In all, 147 columns are marked out in this way in the tables 5-16.

TABLE 5

Parental genotype AB/ab/ab/ab/ab/ab, *representing a set of 4.*
Row divisor : 6

Composition of gamete

Mode of formation	*AB aB ab Ab* / *AB aB ab Ab* / *AB aB ab Ab*	*AB AB ab ab* / *AB AB ab ab* / *Ab aB aB Ab*	*AB AB ab ab* / *Ab aB aB Ab* / *Ab aB aB Ab*	*AB AB Ab aB* / *AB ab Ab aB* / *ab ab aB Ab*	*AB ab AB Ab* / *Ab aB aB Ab* / *aB Ab ab ab*
1	. . 3 .	. . . .	. . . .	. 3 . .	. . . .
2	. . 4 .	. . . .	. . . .	1 1 . .	. . . .
3	. . 2 .	. . 1 1	. . . .	. 2 . .	. . . .
4	. . 4 .	. . . .	. . . .	. . . .	. . 1 1
5	. . 3 .	. . 1 1	. . . .	1 . . .	. . . .
6	. . 1 .	. . 2 2	. . . .	. 1 . .	. . . .
7	. . 3 .	. . . .	. . 1 1	. 1 . .	. . . .
8	. . 3 .	. . . .	. . . .	. 1 . .	. 2 . .
9	. . 2 .	. . 1 1	. . . .	. 1 . .	. 1 . .
10	. . 3 .	. . 1 1	. . . .	. . . .	1 . . .
11	. . 4 .	. . . .	. . . .	. . . .	1 1 . .
12	. . . .	. . 3 3	. . . .	. . . .	. . . .
13	. . 2 .	. . 1 1	. . 1 1	. . . .	. . . .
14	. . 2 .	. . 1 1	. . . .	. . . .	. 2 . .
15	. . 2 .	. . 1 1	. . 1 1	. . . .	. . . .
16	. . 3 .	. . . .	. . 1 1	. . . .	. 1 . .
17	. . 4 .	. . . .	. . . .	. . 1 1	. . . .
18	. . 1 .	. . 2 2	. . . .	. . . .	. 1 . .
19	. . 2 .	. . 1 1	. . . .	. . . .	. 2 . .
20	. . 3 .	. . . .	. . . .	. . . .	. 3 . .
21	. . 3 .	. . 1 .	. . . .	. 1 . .	. . . 1
22	. . 3 .	. . . 1	. . . .	. 1 . .	. . 1 .
23	. . 2 .	. . 2 .	. . . 1	. 1 . .	. . . .
24	. . 2 .	. . . 2	. . 1 .	. 1 . .	. . . .
25	. . 2 .	. . 2 1	. . . .	. . . .	. . . 1
26	. . 2 .	. . 1 2	. . . .	. . . .	. . 1 .
27	. . 3 .	. . . 1	. . 1 .	. . . .	. . . 1
28	. . 3 .	. . 1 .	. . . 1	. . . .	. . 1 .
29	. . 3 .	. . 1 .	. . . .	. . . .	. 1 . 1
30	. . 3 .	. . . 1	. . . .	. . . .	. 1 1 .
31	. . 1 .	. . 3 1	. . . 1	. . . .	. . . .
32	. . 1 .	. . 1 3	. . 1 .	. . . .	. . . .
33	. . 2 .	. . 2 .	. . . 1	. . . .	. 1 . .
34	. . 2 .	. . . 2	. . 1 .	. . . .	. 1 . .
35	. . 2 .	. . 2 1	. . . .	. . 1 .	. . . .
36	. . 2 .	. . 1 2	. . . .	. . . 1	. . . .
37	. . 3 .	. . 1 .	. . . .	. . 1 .	. 1 . .
38	. . 3 .	. . . 1	. . . .	. . . 1	. 1 . .
39	. . 3 .	. . 1 .	. . . 1	. . . 1	. . . .
40	. . 3 .	. . . 1	. . 1 .	. . 1 .	. . . .
		*	* *	* * * *	* * * *

TABLE 6

Parental genotype Ab/aB/ab/ab/ab/ab, *representing a set of 4.*
Row divisor 30

Mode of formation	Composition of gamete																													
	AB AB AB	*aB aB aB*	*ab ab ab*	*Ab Ab Ab*	*AB AB AB Ab*	*AB AB aB aB*	*ab ab aB Ab*	*ab ab Ab Ab*	*AB Ab Ab Ab*	*AB aB aB aB*	*AB aB aB Ab*	*ab aB aB Ab*	*AB AB ab ab*	*AB ab ab ab*	*Ab Ab aB aB*	*aB aB aB Ab*	*AB Ab aB aB*	*ab aB Ab Ab*	*AB ab aB aB*	*AB ab Ab Ab*	*AB Ab ab ab*	*AB aB ab ab*	*AB Ab aB ab*	*AB Ab Ab ab*
1	.	.	6	.	.	.	9	9	.	.	.	.	.	.	.	.	.	6	.	.				
2	.	.	12	.	.	.	4	4	.	.	4	4	.	.	I	I	.	.	.	.				
3	.	.	7	.	.	.	8	8	.	.	.	.	.	I	.	.	.	6	.	.				
4	.	.	12	.	.	.	4	4	.	.	4	4	.	.	.	.	I	I	.	.				
5	.	.	13	.	.	.	3	3	.	.	4	4	.	I	I	I	.	.	.	.				
6	.	.	8	.	.	.	7	7	.	.	.	.	.	2	.	.	.	6	.	.				
7	.	.	13	.	.	.	4	4	.	.	3	3	I	.	I	I	.	.	.	.				
8	.	.	6	.	.	.	9	9	.	.	.	.	.	2	.	.	.	4	.	.				
9	.	.	7	.	.	.	8	8	.	.	.	.	.	2	.	.	.	5	.	.				
10	.	.	13	.	.	.	3	3	.	.	4	4	.	.	.	.	.	I	I	I				
11	.	.	12	.	.	.	4	4	.	.	4	4	.	.	.	.	.	.	I	I				
12	.	.	9	.	.	.	6	6	.	.	.	.	.	3	.	.	.	6	.	.				
13	.	.	14	.	.	.	3	3	.	.	3	3	I	I	I	I	.	.	.	.				
14	.	.	7	.	.	.	8	8	.	.	.	.	.	3	.	.	.	4	.	.				
15	.	.	14	.	.	.	3	3	.	.	3	3	.	.	.	.	I	I	I	I				
16	.	.	13	.	.	.	4	4	.	.	3	3	.	.	.	.	I	.	I	I				
17	.	.	12	.	.	.	4	4	.	.	4	4	I	I	.	.	.	.	.	.				
18	.	.	8	.	.	.	7	7	.	.	.	.	.	3	.	.	.	5	.	.				
19	.	.	7	.	.	.	8	8	.	.	.	.	.	3	.	.	.	4	.	.				
20	.	.	6	.	.	.	9	9	.	.	.	.	.	3	.	.	.	3	.	.				
21	.	.	9	.	.	.	11	3	.	.	.	3	.	.	I	.	.	2	.	I				
22	.	.	9	.	.	.	3	11	.	.	3	.	.	.	.	I	.	2	I	.				
23	.	.	10	.	.	.	10	3	.	.	.	2	.	.	I	.	.	2	.	2				
24	.	.	10	.	.	.	3	10	.	.	2	.	.	.	.	I	.	2	2	.				
25	.	.	10	.	.	.	10	2	.	.	.	3	.	I	I	.	.	2	.	I				
26	.	.	10	.	.	.	2	10	.	.	3	.	.	I	.	I	.	2	I	.				
27	.	.	13	.	.	.	4	3	.	.	3	4	.	.	.	.	I	I	I	.				
28	.	.	13	.	.	.	3	4	.	.	4	3	.	.	.	.	I	I	.	I				
29	.	.	9	.	.	.	11	3	.	.	.	3	.	I	I	.	.	I	.	I				
30	.	.	9	.	.	.	3	11	.	.	3	.	.	I	.	I	.	I	I	.				
31	.	.	11	.	.	.	9	2	.	.	.	2	.	I	I	.	.	2	.	2				
32	.	.	11	.	.	.	2	9	.	.	2	.	.	I	.	I	.	2	2	.				
33	.	.	10	.	.	.	10	3	.	.	.	2	.	I	I	.	.	I	.	2				
34	.	.	10	.	.	.	3	10	.	.	2	.	.	I	.	I	.	I	2	.				
35	.	.	10	.	.	.	10	2	.	.	.	3	.	I	.	.	.	2	.	2				
36	.	.	10	.	.	.	2	10	.	.	3	.	.	I	.	.	.	2	2	.				
37	.	.	9	.	.	.	11	3	.	.	.	3	.	I	.	.	.	I	.	2				
38	.	.	9	.	.	.	3	11	.	.	3	.	.	I	.	.	.	I	2	.				
39	.	.	13	.	.	.	3	4	.	.	4	3	I	I	I	.	.	.	.	.				
40	.	.	13	.	.	.	4	3	.	.	3	4	I	I	.	I	.	.	.	.				
							*			*	*		*	*	*	*		*	*	*	*			

TABLE 7

Parental genotype AB/AB/ab/ab/ab/ab, *representing a set of 4.*
Row divisor : 15

Composition of gamete

Mode of formation	*AB AB AB*	*aB aB aB*	*ab ab ab*	*Ab Ab Ab*	*AB AB Ab*	*AB aB aB*	*ab ab aB*	*ab Ab Ab*	*AB Ab Ab*	*aB aB aB*	*ab aB aB*	*ab Ab Ab*	*AB AB ab*	*AB ab ab*	*Ab Ab aB*	*aB Ab Ab*	*AB Ab aB*	*ab Ab aB*	*AB aB ab*	*AB Ab ab*
1	.	.	3	.	.	.	.	.	.	.	.	.	3	9	.	.	.	.	.	.
2	1	.	6	.	.	.	.	.	.	.	.	.	4	4	.	.	.	.	.	.
3	.	.	1	.	.	.	2	2	.	.	.	.	1	5	.	.	.	.	2	2
4	1	.	6	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	4	4
5	.	.	3	.	1	1	3	3	.	.	.	.	3	1	.	.	.	.	.	.
6	.	.	.	.	.	.	2	2	.	.	1	1	.	3	.	.	.	2	2	2
7	.	.	3	.	.	.	.	.	1	1	3	3	1	3	.	.	.	.	.	.
8	.	.	3	.	.	.	.	.	.	.	.	.	1	3	.	.	2	6	.	.
9	.	.	1	.	.	.	2	2	.	.	.	.	.	2	.	.	1	3	2	2
10	.	.	3	.	1	1	3	3	.	.	.	.	.	.	.	.	3	1	.	.
11	1	.	6	.	.	.	.	.	.	.	.	.	.	.	.	.	4	4	.	.
12	.	.	.	.	.	.	.	.	.	.	3	3	.	3	.	.	.	6	.	.
13	.	1	1	1	.	.	2	2	.	.	2	2	1	1	1	1	.	.	.	.
14	.	.	1	.	.	.	2	2	.	.	.	.	1	1	2	2	.	4	.	.
15	.	1	1	1	.	.	2	2	.	.	2	2	.	.	.	.	1	1	1	1
16	.	.	3	.	.	.	.	.	1	1	3	3	.	.	.	.	1	3	.	.
17	1	.	6	.	.	.	.	.	.	.	.	.	.	.	4	4	.	.	.	.
18	.	.	.	.	.	.	2	2	.	.	1	1	.	1	1	1	.	4	1	1
19	.	.	1	.	.	.	2	2	.	.	.	.	.	.	1	1	1	5	1	1
20	.	.	3	.	.	.	.	.	.	.	.	.	.	.	.	.	3	9	.	.
21	.	.	3	.	1	.	3	.	.	.	.	.	1	3	.	.	.	.	1	3
22	.	.	3	.	.	1	.	3	.	.	.	.	1	3	.	.	.	.	3	1
23	.	.	1	.	.	.	4	.	1	.	1	2	.	2	.	.	.	.	2	2
24	.	.	1	.	.	.	.	4	.	1	2	1	.	2	.	.	.	.	2	2
25	.	.	1	.	.	.	4	2	1	.	1	.	1	1	.	.	1	1	.	2
26	.	.	1	.	.	.	2	4	.	1	.	1	1	1	.	.	1	1	2	.
27	.	.	3	.	.	1	.	3	1	.	3	.	.	.	.	.	.	.	1	3
28	.	.	3	.	1	.	3	.	.	1	.	3	.	.	.	.	.	.	3	1
29	.	.	3	.	1	.	3	.	.	.	.	.	.	.	.	.	1	3	1	3
30	.	.	3	.	.	1	.	3	.	.	.	.	.	.	.	.	1	3	3	1
31	.	.	.	1	.	.	3	1	.	.	3	1	.	1	1	.	.	2	.	2
32	.	1	.	.	.	.	1	3	.	.	1	3	.	1	.	1	.	2	2	.
33	.	.	1	.	.	.	4	.	1	.	1	2	.	.	1	1	.	2	1	1
34	.	.	1	.	.	.	.	4	.	1	2	1	.	.	1	1	.	2	1	1
35	.	.	1	.	.	.	4	2	1	.	1	.	.	.	2	.	2	2	.	.
36	.	.	1	.	.	.	2	4	.	1	.	1	.	.	.	2	2	2	.	.
37	.	.	3	.	1	.	3	.	.	.	.	.	.	.	3	1	1	3	.	.
38	.	.	3	.	.	1	.	3	.	.	.	.	.	.	1	3	1	3	.	.
39	.	.	3	.	1	.	3	.	.	1	.	3	.	.	1	3	.	.	.	.
40	.	.	3	.	.	1	.	3	1	.	3	.	.	.	3	1	.	.	.	.
	*	*	*	*	*	*	*		*	*	*		*		*		*	*	*	*

TABLE 8

Parental genotype Ab/Ab/aB/aB/ab/ab, *representing a set of 4.*
Row divisor : 90

Composition of gamete

Mode of formation	AB AB AB	AB aB aB	aB ab ab	ab Ab Ab	AB AB Ab	AB aB aB	ab ab aB	ab Ab Ab	AB Ab Ab	AB aB aB	ab aB aB	ab Ab Ab	AB AB ab	AB ab ab	Ab aB aB	aB aB Ab	AB Ab aB	ab aB Ab	AB ab ab	Ab aB Ab
1	.	.	.	.	.	.	9	9	.	.	9	9	.	.	9	9	.	36	.	.
2	.	6	6	6	.	.	12	12	.	.	12	12	.	.	12	12	.	.	.	.
3	.	.	2	.	.	.	11	11	.	.	5	5	.	2	7	7	2	30	4	4
4	.	6	6	6	.	.	12	12	.	.	12	12	.	.	.	.	12	12	.	.
5	.	3	12	3	.	.	6	6	3	3	15	15	.	6	9	9	.	.	.	.
6	.	.	5	.	.	.	11	11	.	.	2	2	1	6	5	5	4	26	6	6
7	.	3	12	3	3	3	15	15	.	.	6	6	6	.	9	9	.	.	.	.
8	.	.	.	.	.	.	9	9	.	.	9	9	.	12	3	3	.	24	6	6
9	.	.	2	.	.	.	11	11	.	.	5	5	.	8	4	4	2	24	7	7
10	.	3	12	3	.	.	6	6	3	3	15	15	.	.	.	.	.	6	9	9
11	.	6	6	6	.	.	12	12	.	.	12	12	.	.	.	.	.	.	12	12
12	.	.	9	.	.	.	9	9	.	.	.	.	3	12	3	3	6	24	6	6
13	1	1	19	1	2	2	8	8	2	2	8	8	7	7	7	7	.	.	.	.
14	.	.	2	.	.	.	11	11	.	.	5	5	2	16	3	3	.	16	8	8
15	1	1	19	1	2	2	8	8	2	2	8	8	.	.	.	.	7	7	7	7
16	.	3	12	3	3	3	15	15	.	.	6	6	.	.	.	.	6	.	9	9
17	.	6	6	6	.	.	12	12	.	.	12	12	12	12	.	.	.	.	.	.
18	.	.	5	.	.	.	11	11	.	.	2	2	2	13	3	3	3	19	8	8
19	.	.	2	.	.	.	11	11	.	.	5	5	1	15	2	2	1	17	9	9
20	.	.	.	.	.	.	9	9	.	.	9	9	.	18	.	.	.	18	9	9
21	.	.	3	3	.	.	21	6	3	.	12	6	.	.	9	3	3	15	.	6
22	.	3	3	.	.	.	6	21	.	3	6	12	.	.	3	9	3	15	6	.
23	.	.	7	1	1	.	22	8	4	.	7	2	2	.	6	2	6	14	.	8
24	.	1	7	.	.	1	8	22	.	4	2	7	2	.	2	6	6	14	8	.
25	.	.	7	1	1	.	22	2	4	.	7	8	.	4	7	3	2	10	5	7
26	.	1	7	.	.	1	2	22	.	4	8	7	.	4	3	7	2	10	7	5
27	.	3	12	3	3	.	15	6	.	3	6	15	.	.	.	.	9	9	6	.
28	.	3	12	3	.	3	6	15	3	.	15	6	.	.	.	.	9	9	.	6
29	.	.	3	3	.	.	21	6	3	.	12	6	.	6	6	.	3	9	3	9
30	.	3	3	.	.	.	6	21	.	3	6	12	.	6	.	6	3	9	9	3
31	.	.	12	.	3	.	21	3	3	.	3	3	2	5	5	2	4	10	4	10
32	.	.	12	.	.	3	3	21	.	3	3	3	2	5	2	5	4	10	10	4
33	.	.	7	1	1	.	22	8	4	.	7	2	3	7	4	.	5	7	2	10
34	.	1	7	.	.	1	8	22	.	4	2	7	3	7	.	4	5	7	10	2
35	.	.	7	1	1	.	22	2	4	.	7	8	2	6	.	2	.	8	6	14
36	.	1	7	.	.	1	2	22	.	4	8	7	2	6	2	.	.	8	14	6
37	.	.	3	3	.	.	21	6	3	.	12	6	3	9	.	.	.	6	3	15
38	.	3	3	.	.	.	6	21	.	3	6	12	3	9	.	.	.	6	15	3
39	.	3	12	3	.	3	6	15	3	.	15	6	9	9	6	.	.	.	.	.
40	.	3	12	3	3	.	15	6	.	3	6	15	9	9	.	6	.	.	.	.
	*	*	*		*	*	*	*	*	*	*	*	*		*		*	*		*

TABLE 9

Parental genotype AB/Ab/aB/ab/ab/ab, *representing a set of 4.*
Row divisor : 120

Mode of formation	Composition of gamete																															
	AB AB AB	*aB aB aB*	*ab ab ab*	*Ab Ab Ab*	*AB AB Ab*	*AB AB aB*	*ab ab aB*	*ab ab Ab*	*AB Ab Ab*	*AB aB aB*	*ab ab aB*	*ab ab Ab*	*AB AB ab*	*AB ab ab*	*Ab Ab aB*	*aB aB Ab*	*AB Ab aB*	*ab aB Ab*	*AB aB ab*	*AB Ab ab*	*ab aB aB*	*ab Ab ab*	*AB aB Ab*	*AB Ab aB*	*ab aB ab*	*ab Ab Ab*	*AB Ab Ab*	*aB aB aB*	*ab ab aB*	*ab Ab Ab*	*AB aB aB*	*AB ab ab*	*Ab aB ab*
1	.	.	6	.	.	.	18	18	.	.	.	.	.	18	.	.	6	18	18	18												
2	.	.	24	.	4	4	12	12	4	4	12	12	12	12	4	4	.	.	.	.												
3	.	.	6	.	.	.	14	14	.	.	4	4	2	20	2	2	4	24	12	12												
4	.	.	24	.	4	4	12	12	4	4	12	12	.	.	.	.	4	4	12	12												
5	1	3	21	3	3	3	15	15	1	1	9	9	13	9	7	7	.	.	.	.												
6	.	.	4	.	.	.	14	14	.	.	6	6	2	18	4	4	2	26	10	10												
7	1	3	21	3	1	1	9	9	3	3	15	15	9	13	7	7	.	.	.	.												
8	.	.	6	.	.	.	18	18	.	.	.	.	2	12	6	6	4	24	12	12												
9	.	.	6	.	.	.	14	14	.	.	4	4	3	17	5	5	3	27	9	9												
10	1	3	21	3	3	3	15	15	1	1	9	9	.	.	.	.	13	9	7	7												
11	.	.	24	.	4	4	12	12	4	4	12	12	.	.	.	.	12	12	4	4												
12	.	.	.	.	.	.	18	18	.	.	6	6	.	12	6	6	.	24	12	12												
13	.	4	16	4	2	2	14	14	2	2	14	14	8	8	8	8	.	.	.	.												
14	.	.	6	.	.	.	14	14	.	.	4	4	.	10	4	4	6	34	10	10												
15	.	4	16	4	2	2	14	14	2	2	14	14	.	.	.	.	8	8	8	8												
16	1	3	21	3	1	1	9	9	3	3	15	15	.	.	.	.	9	13	7	7												
17	.	.	24	.	4	4	12	12	4	4	12	12	4	4	12	12	.	.	.	.												
18	.	.	4	.	.	.	14	14	.	.	6	6	1	13	5	5	3	31	9	9												
19	.	.	6	.	.	.	14	14	.	.	4	4	2	12	6	6	4	32	8	8												
20	.	.	6	.	.	.	18	18	.	.	.	.	3	9	9	9	3	27	9	9												
21	.	.	12	.	2	.	30	6	6	.	6	6	3	9	3	1	5	9	7	15												
22	.	.	12	.	.	2	6	30	.	6	6	6	3	9	1	3	5	9	15	7												
23	.	.	10	2	2	.	28	4	4	.	10	8	2	10	6	2	2	10	6	14												
24	.	2	10	.	.	2	4	28	.	4	8	10	2	10	2	6	2	10	14	6												
25	.	.	10	2	2	.	28	8	4	.	10	4	3	7	5	1	5	13	3	15												
26	.	2	10	.	.	2	8	28	.	4	4	10	3	7	1	5	5	13	15	3												
27	1	3	21	3	1	3	9	15	3	1	15	9	.	.	.	.	7	7	9	13												
28	1	3	21	3	3	1	15	9	1	3	9	15	.	.	.	.	7	7	13	9												
29	.	.	12	.	2	.	30	6	6	.	6	6	4	6	6	4	4	12	4	12												
30	.	.	12	.	.	2	6	30	.	6	6	6	4	6	4	6	4	12	12	4												
31	.	.	6	2	.	.	30	8	6	.	12	8	2	6	6	2	4	12	4	12												
32	.	2	6	.	.	.	8	30	.	6	8	12	2	6	2	6	4	12	12	4												
33	.	.	10	2	2	.	28	4	4	.	10	8	1	5	7	3	3	15	5	13												
34	.	2	10	.	.	2	4	28	.	4	8	10	1	5	3	7	3	15	13	5												
35	.	.	10	2	2	.	28	8	4	.	10	4	2	6	10	2	6	14	2	10												
36	.	2	10	.	.	2	8	28	.	4	4	10	2	6	2	10	6	14	10	2												
37	.	.	12	.	2	.	30	6	6	.	6	6	1	3	9	3	7	15	5	9												
38	.	.	12	.	.	2	6	30	.	6	6	6	1	3	3	9	7	15	9	5												
39	1	3	21	3	3	1	15	9	1	3	9	15	7	7	9	13	.	.	.	.												
40	1	3	21	3	1	3	9	15	3	1	15	9	7	7	13	9	.	.	.	.												
	*	*	*	*	*	*	*		*	*	*	*	*	*	*						*	*										

TABLE 10

Parental genotype AB/AB/AB/ab/ab/ab, *representing a set of 2.*
Row divisor : 20

Composition of gamete

Mode of formation	*AB aB ab Ab* / *AB aB ab Ab* / *AB aB ab Ab*	*AB AB ab ab* / *AB AB ab ab* / *Ab aB aB Ab*	*AB AB ab ab* / *Ab aB aB Ab* / *Ab aB aB Ab*	*AB AB ab ab* / *AB ab ab ab* / *ab ab ab ab*	*AB AB Ab aB* / *Ab Ab aB aB* / *aB Ab aB Ab*	*AB ab AB Ab* / *aB aB ab ab* / *aB Ab ab Ab*
1	1 . 1 .	. . . .	. . . .	9 9 . .	. . . .	. . . .
2	4 . 4 .	. . . .	. . . .	6 6 . .	. . . .	. . . .
3	. . . .	1 1 1 1	. . . .	4 4 . .	. . 4 4	. . 6 6
4	4 . 4 .	. . . .	. . . .	. . . .	. . 6 6	. . . .
5	1 . 1 .	3 3 3 3	. . . .	3 3 . .	. . . .	. . . .
6	. . . .	. . . .	1 1 1 1	2 2 . .	2 2 4 4	. . . .
7	1 . 1 .	. . . .	3 3 3 3	3 3 . .	. . . .	. . . .
8	1 . 1 .	. . . .	. . . .	3 3 . .	6 6 . .	. . . .
9	. . . .	1 1 1 1	. . . .	1 1 . .	3 3 4 4	. . . .
10	1 . 1 .	3 3 3 3	. . . .	. . . .	3 3 . .	. . . .
11	4 . 4 .	. . . .	. . . .	. . . .	6 6 . .	. . . .
12	. 1 . 1	. . . .	. . . .	. . 3 3	. . 6 6	. . . .
13	. 2 . 2	1 1 1 1	1 1 1 1	2 2 2 2	. . . .	. . . .
14	. . . .	1 1 1 1	. . . .	2 2 4 4	2 2 . .	. . . .
15	. 2 . 2	1 1 1 1	1 1 1 1	. . . .	2 2 2 2	. . . .
16	1 . 1 .	. . . .	3 3 3 3	. . . .	3 3 . .	. . . .
17	4 . 4 .	. . . .	. . . .	. . 6 6	. . . .	. . . .
18	. . . .	. . . .	1 1 1 1	1 1 2 2	3 3 2 2	. . . .
19	. . . .	1 1 1 1	. . . .	. . 2 2	4 4 2 2	. . . .
20	1 . 1 .	. . . .	. . . .	. . . .	9 9 . .	. . . .
21	1 . 1 .	3 . 3 .	. . . .	3 3 . .	. . 3 3	. . . .
22	1 . 1 .	. 3 . 3	. . . .	3 3 . .	. . 3 3	. . . .
23	. . . .	2 . 2 .	2 1 2 1	1 1 . .	. . 4 4	. . . .
24	. . . .	. 2 . 2	1 2 1 2	1 1 . .	. . 4 4	. . . .
25	. . . .	2 1 2 1	2 . 2 .	2 2 . .	2 2 1 1	. . . .
26	. . . .	1 2 1 2	. 2 . 2	2 2 . .	2 2 1 1	. . . .
27	1 . 1 .	. 3 . 3	3 . 3 .	. . . .	. . 3 3	. . . .
28	1 . 1 .	3 . 3 .	. 3 . 3	. . . .	. . 3 3	. . . .
29	1 . 1 .	3 . 3 .	. . . .	. . . .	3 3 3 3	. . . .
30	1 . 1 .	. 3 . 3	. . . .	. . . .	3 3 3 3	. . . .
31	. 1 . 1	. . . .	3 . 3 .	1 1 1 1	2 2 2 2	. . . .
32	. 1 . 1	. . . .	. 3 . 3	1 1 1 1	2 2 2 2	. . . .
33	. . . .	2 . 2 .	2 1 2 1	. . 2 2	1 1 2 2	. . . .
34	. . . .	. 2 . 2	1 2 1 2	. . 2 2	1 1 2 2	. . . .
35	. . . .	2 1 2 1	2 . 2 .	. . 1 1	4 4 . .	. . . .
36	. . . .	1 2 1 2	. 2 . 2	. . 1 1	4 4 . .	. . . .
37	1 . 1 .	3 . 3 .	. . . .	. . 3 3	3 3 . .	. . . .
38	1 . 1 .	. 3 . 3	. . . .	. . 3 3	3 3 . .	. . . .
39	1 . 1 .	3 . 3 .	. 3 . 3	. . 3 3	. . . .	. . . .
40	1 . 1 .	. 3 . 3	3 . 3 .	. . 3 3	. . . .	. . . .
	* *	* *	* *	*	*	* *

TABLE 11

Parental genotype AB/AB/Ab/aB/ab/ab, *representing a set of 2.*
Row divisor : 180

Composition of gamete

Mode of formation	*AB AB Ab* / *AB aB ab*	*AB aB ab* / *AB aB ab*	*Ab Ab ab* / *Ab aB ab*	*Ab Ab Ab* / *Ab aB ab*
1	. . .	9 9 9	. . .	18 18 .
2	12 . 12	12 12 12	12 12 12	24 24 6
3	1 . 1	4 4 4	4 4 4	19 19 4
4	12 . 12	12 12 12	12 12 12	. . .
5	9 6 9	15 15 15	6 6 6	21 21 12
6	. 1 .	4 4 4	4 4 4	14 14 11
7	9 6 9	6 6 6	15 15 15	21 21 12
8	. . .	9 9 9	. . .	12 12 12
9	1 . 1	4 4 4	4 4 4	16 16 10
10	9 6 9	15 15 15	6 6 6	. . .
11	12 . 12	12 12 12	12 12 12	. . .
12	. . .	. . .	9 9 9	12 12 12
13	4 10 4	11 11 11	11 11 11	16 16 16
14	1 . 1	4 4 4	4 4 4	9 9 12
15	4 10 4	11 11 11	11 11 11	. . .
16	9 6 9	6 6 6	15 15 15	. . .
17	12 . 12	12 12 12	12 12 12	6 6 24
18	. 1 .	4 4 4	4 4 4	9 9 15
19	1 . 1	4 4 4	4 4 4	11 11 14
20	. . .	9 9 9	. . .	9 9 18
21	3 . 3	21 3 21	12 3 12	12 12 3
22	3 . 3	3 21 3	3 12 3	12 12 3
23	2 2 2	16 1 16	16 4 16	10 10 7
24	2 2 2	1 16 1	4 16 4	10 10 7
25	2 2 2	16 4 16	16 1 16	11 11 5
26	2 2 2	4 16 4	1 16 1	11 11 5
27	9 6 9	6 15 6	15 6 15	. . .
28	9 6 9	15 6 15	6 15 6	. . .
29	3 . 3	21 3 21	12 3 12	9 9 9
30	3 . 3	3 21 3	3 12 3	9 9 9
31	. 3 .	12 3 12	21 3 21	8 8 8
32	. 3 .	3 12 3	3 21 3	8 8 8
33	2 2 2	16 1 16	16 4 16	5 5 11
34	2 2 2	1 16 1	4 16 4	5 5 11
35	2 2 2	16 4 16	16 1 16	7 7 10
36	2 2 2	4 16 4	1 16 1	7 7 10
37	3 . 3	21 3 21	12 3 12	3 3 12
38	3 . 3	3 21 3	3 12 3	3 3 12
39	9 6 9	15 6 15	6 15 6	12 12 21
40	9 6 9	6 15 6	15 6 15	12 12 21
	* *	* *	* *	* *

Mode of formation	*AB ab AB Ab* / *aB aB ab*	*AB Ab aB ab* / *aB aB ab*
1	18 18 36	36
2	. . .	.
3	22 22 28	28
4	6 6 24	24
5	. . .	.
6	18 18 30	30
7	. . .	.
8	24 24 24	24
9	25 25 22	22
10	21 21 12	12
11	24 24 6	6
12	24 24 24	24
13	. . .	.
14	32 32 20	20
15	16 16 16	16
16	21 21 12	12
17	. . .	.
18	23 23 26	26
19	30 30 18	18
20	27 27 18	18
21	12 12 21	21
22	12 12 21	21
23	10 10 22	22
24	10 10 22	22
25	18 18 15	15
26	18 18 15	15
27	12 12 21	21
28	12 12 21	21
29	15 15 15	15
30	15 15 15	15
31	16 16 16	16
32	16 16 16	16
33	15 15 18	18
34	15 15 18	18
35	22 22 10	10
36	22 22 10	10
37	21 21 12	12
38	21 21 12	12
39	. . .	.
40	. . .	.
	* *	

TABLE 12

Parental genotype Ab/Ab/aB/ab/ab/ab, *representing a set of 4. An interchange of loci accompanied by an interchange of paired conjugate modes generates a conjugate set of 4. Row divisor : 60*

Composition of gamete

Mode of formation	*AB AB AB*	*aB aB aB*	*ab ab ab*	*Ab Ab Ab*	*AB AB AB*	*AB aB aB*	*ab ab aB*	*ab aB Ab*	*AB Ab Ab*	*AB aB aB*	*ab aB Ab*	*ab aB Ab*	*AB AB ab*	*AB ab ab*	*Ab Ab aB*	*aB aB Ab*	*AB ab aB*	*AB aB Ab*	*AB aB ab*	*AB aB ab*
1	.	.	3	.	.	.	9	18	.	.	.	9	.	.	3	.	.	18	.	.
2	.	.	12	4	.	.	6	12	.	.	6	12	.	.	4	4	.	.	.	.
3	.	.	5	.	.	.	7	18	.	.	.	7	.	2	3	.	.	16	.	2
4	.	.	12	4	.	.	6	12	.	.	6	12	.	.	.	.	4	4	.	.
5	.	.	15	3	.	.	3	9	1	.	6	13	.	3	3	4	.	.	.	.
6	.	.	7	.	.	.	5	18	.	.	.	5	.	4	3	.	.	14	.	4
7	.	.	15	3	1	.	6	13	.	.	3	9	3	.	4	3	.	.	.	.
8	.	.	3	.	.	.	9	18	.	.	.	9	.	6	1	.	.	12	.	2
9	.	.	5	.	.	.	7	18	.	.	.	7	.	5	2	.	.	13	.	3
10	.	.	15	3	.	.	3	9	1	.	6	13	.	.	.	.	.	3	4	3
11	.	.	12	4	.	.	6	12	.	.	6	12	.	.	.	.	.	.	4	4
12	.	.	9	.	.	.	3	18	.	.	.	3	.	6	3	.	.	12	.	6
13	.	.	18	2	1	.	3	10	1	.	3	10	3	3	3	3	.	.	.	.
14	.	.	5	.	.	.	7	18	.	.	.	7	.	8	1	.	.	10	.	4
15	.	.	18	2	1	.	3	10	1	.	3	10	.	.	.	.	3	3	3	3
16	.	.	15	3	1	.	6	13	.	.	3	9	.	.	.	.	3	.	3	4
17	.	.	12	4	.	.	6	12	.	.	6	12	4	4	.	.	.	.	.	.
18	.	.	7	.	.	.	5	18	.	.	.	5	.	7	2	.	.	11	.	5
19	.	.	5	.	.	.	7	18	.	.	.	7	.	8	1	.	.	10	.	4
20	.	.	3	.	.	.	9	18	.	.	.	9	.	9	.	.	.	9	.	3
21	.	.	9	3	.	.	15	9	1	.	.	9	.	.	4	.	.	7	.	3
22	.	.	6	.	.	.	3	24	.	.	3	10	.	.	1	3	1	6	3	.
23	.	.	12	2	.	.	12	10	2	.	.	6	.	.	4	.	.	6	.	6
24	.	.	8	.	.	.	3	24	.	.	1	8	1	.	1	2	2	6	4	.
25	.	.	12	2	.	.	12	6	2	.	.	10	.	3	3	.	.	7	.	3
26	.	.	8	.	.	.	1	24	.	.	3	8	.	2	1	3	1	4	3	2
27	.	.	15	3	1	.	6	9	.	.	3	13	.	.	.	.	3	4	3	.
28	.	.	15	3	.	.	3	13	1	.	6	9	.	.	.	.	4	3	.	3
29	.	.	9	3	.	.	15	9	1	.	.	9	.	3	3	.	.	4	.	4
30	.	.	6	.	.	.	3	24	.	.	3	10	.	3	.	3	1	3	3	1
31	.	.	15	1	.	.	9	7	3	.	.	7	.	3	3	.	.	6	.	6
32	.	.	10	.	.	.	1	24	.	.	1	6	1	2	1	2	2	4	4	2
33	.	.	12	2	.	.	12	10	2	.	.	6	.	3	3	.	.	3	.	7
34	.	.	8	.	.	.	3	24	.	.	1	8	1	3	.	2	2	3	4	1
35	.	.	12	2	.	.	12	6	2	.	.	10	.	4	.	.	.	6	.	6
36	.	.	8	.	.	.	1	24	.	.	3	8	1	2	1	.	.	4	6	2
37	.	.	9	3	.	.	15	9	1	.	.	9	.	4	.	.	.	3	.	7
38	.	.	6	.	.	.	3	24	.	.	3	10	1	3	.	.	.	3	6	1
39	.	.	15	3	.	.	3	13	1	.	6	9	4	3	3	.	.	.	.	.
40	.	.	15	3	1	.	6	9	.	.	3	13	3	4	.	3	.	.	.	.
			*	*	*				*		*		*	*	*	*	*	*	*	*

TABLE 13

Parental genotype AB/Ab/ab/ab/ab/ab, *representing a set of 4. An interchange of loci accompanied by an interchange of paired conjugate modes generates a conjugate set of 4. Row divisor : 30*

| Mode of formation | Composition of gamete | | |

TABLE 14

Parental genotype AB/Ab/Ab/ab/ab/ab, *representing a set of 4. An interchange of loci accompanied by an interchange of paired conjugate modes generates a conjugate set of 4. Row divisor : 60*

Mode of formation	Composition of gamete																															
	AB AB AB	*aB aB aB*	*ab ab ab*	*Ab Ab Ab*	*AB AB Ab*	*AB aB aB*	*ab aB aB*	*ab Ab Ab*	*AB Ab Ab*	*AB aB aB*	*ab aB aB*	*ab Ab Ab*	*AB AB ab*	*AB ab ab*	*Ab aB aB*	*aB Ab Ab*	*AB Ab aB*	*ab aB Ab*	*AB aB ab*	*Ab aB ab*	*AB Ab ab*	*aB aB ab*	*AB Ab aB*	*ab aB Ab*	*AB aB aB*	*Ab Ab Ab*	*AB Ab Ab*	*aB aB Ab*	*AB aB Ab*	*ab aB Ab*	*AB Ab Ab*	*aB aB Ab*
1	.	.	3	.	.	.	.	18	3	.	.	9	.	9	.	.	.	.	.	18												
2	.	.	12	4	4	.	.	12	4	.	.	12	6	6	.	.	.	.	.	.												
3	.	.	2	1	.	.	1	15	2	.	.	12	.	8	1	.	.	4	.	14												
4	.	.	12	4	4	.	.	12	4	.	.	12	.	.	.	.	.	.	6	6												
5	.	.	9	7	4	.	3	15	1	.	.	9	6	3	3	.	.	.	.	.												
6	.	.	1	2	.	.	2	12	1	.	.	15	.	7	2	.	.	8	.	10												
7	.	.	9	7	1	.	.	9	4	.	3	15	3	6	.	3	.	.	.	.												
8	.	.	3	.	.	.	.	18	3	.	.	9	.	3	6	.	.	6	.	12												
9	.	.	2	1	.	.	1	15	2	.	.	12	.	5	4	.	.	7	.	11												
10	.	.	9	7	4	.	3	15	1	.	.	9	.	.	.	.	6	3	.	3												
11	.	.	12	4	4	.	.	12	4	.	.	12	.	.	.	.	6	6	.	.												
12	.	.	.	3	.	.	3	9	.	.	.	18	.	6	3	.	.	12	.	6												
13	.	.	6	10	1	.	3	12	1	.	3	12	3	3	3	3	.	.	.	.												
14	.	.	2	1	.	.	1	15	2	.	.	12	.	2	7	.	.	10	.	8												
15	.	.	6	10	1	.	3	12	1	.	3	12	.	.	.	.	3	3	3	3												
16	.	.	9	7	1	.	.	9	4	.	3	15	.	.	.	.	3	6	3	.												
17	.	.	12	4	4	.	.	12	4	.	.	12	.	.	6	6	.	.	.	.												
18	.	.	1	2	.	.	2	12	1	.	.	15	.	4	5	.	.	11	.	7												
19	.	.	2	1	.	.	1	15	2	.	.	12	.	2	7	.	.	10	.	8												
20	.	.	3	.	.	.	.	18	3	.	.	9	.	.	9	.	.	9	.	9												
21	.	.	9	3	.	.	3	9	9	.	.	9	.	6	.	.	.	3	.	9												
22	.	.	3	1	1	.	.	21	1	.	.	15	3	3	.	.	3	.	3	6												
23	.	.	6	6	.	.	6	6	6	.	.	12	.	6	.	.	.	6	.	6												
24	.	.	2	2	.	.	.	18	1	.	1	18	1	3	.	2	2	.	4	6												
25	.	.	6	6	.	.	6	12	6	.	.	6	.	3	3	.	.	3	.	9												
26	.	.	2	2	1	.	1	18	.	.	.	18	3	2	1	.	3	4	3	2												
27	.	.	9	7	1	.	.	15	4	.	3	9	.	.	.	.	3	.	3	6												
28	.	.	9	7	4	.	3	9	1	.	.	15	.	.	.	.	.	3	6	3												
29	.	.	9	3	.	.	3	9	9	.	.	9	.	3	3	.	.	6	.	6												
30	.	.	3	1	1	.	.	21	1	.	.	15	3	.	3	.	3	3	3	3												
31	.	.	3	9	.	.	9	9	3	.	.	9	.	3	3	.	.	6	.	6												
32	.	.	1	3	.	.	1	15	.	.	1	21	1	2	1	2	2	4	4	2												
33	.	.	6	6	.	.	6	6	6	.	.	12	.	3	3	.	.	9	.	3												
34	.	.	2	2	.	.	.	18	1	.	1	18	1	.	3	2	2	3	4	3												
35	.	.	6	6	.	.	6	12	6	.	.	6	.	.	6	.	.	6	.	6												
36	.	.	2	2	1	.	1	18	.	.	.	18	.	2	1	3	6	4	.	2												
37	.	.	9	3	.	.	3	9	9	.	.	9	.	.	6	.	.	9	.	3												
38	.	.	3	1	1	.	.	21	1	.	.	15	.	.	3	3	6	3	.	3												
39	.	.	9	7	4	.	3	9	1	.	.	15	.	3	3	6	.	.	.	.												
40	.	.	9	7	1	.	.	15	4	.	3	9	3	.	6	3	.	.	.	.												
					*		*	*	*		*	*	*	*		*	*		*												

TABLE 15

Parental genotype AB/AB/Ab/ab/ab/ab, *representing a set of 4. An interchange of loci accompanied by an interchange of paired conjugate modes generates a conjugate set of 4. Row divisor : 60*

Mode of formation	Composition of gamete																													
	AB AB Ab	*aB aB aB*	*ab ab ab*	*Ab Ab Ab*	*AB AB Ab*	*AB ab aB*	*AB ab aB*	*ab ab Ab*	*AB AB Ab*	*AB ab aB*	*AB ab aB*	*ab ab Ab*	*AB AB AB*	*ab ab ab*	*Ab aB aB*	*aB aB Ab*	*AB ab ab*	*AB ab ab*	*Ab aB aB*	*aB aB Ab*	*AB Ab aB*	*ab aB Ab*	*AB aB ab*	*AB Ab ab*	*AB Ab aB*	*ab aB aB*	*AB Ab ab*	*AB Ab ab*	*AB Ab aB*	*ab aB Ab*
1	.	.	3	.	3	.	.	9	.	.	.	.	9	18	.	.	.	.	.	18										
2	4	.	12	.	4	.	.	6	4	.	.	6	12	12	.	.	.	.	.	.										
3	.	.	1	.	1	.	2	7	2	.	.	4	5	12	.	.	2	4	4	16										
4	4	.	12	.	4	.	.	6	4	.	.	6	.	.	.	.	.	.	12	12										
5	1	.	6	3	7	3	6	12	1	.	.	3	9	6	3	.	.	.	.	.										
6	.	.	.	1	.	.	2	4	2	.	1	7	2	8	2	1	2	8	6	14										
7	1	.	6	3	1	.	.	3	7	3	6	12	6	9	.	3	.	.	.	.										
8	.	.	3	.	3	.	.	9	.	.	.	.	3	6	6	.	6	12	.	12										
9	.	.	1	.	1	.	2	7	2	.	.	4	2	6	3	.	5	10	4	13										
10	1	.	6	3	7	3	6	12	1	.	.	3	.	.	.	.	9	6	.	3										
11	4	.	12	.	4	.	.	6	4	.	.	6	.	.	.	.	12	12	.	.										
12	.	.	.	3	.	.	.	.	.	.	3	9	.	6	6	3	.	12	6	12										
13	.	2	2	8	2	1	4	7	2	1	4	7	5	5	5	5	.	.	.	.										
14	.	.	1	.	1	.	2	7	2	.	.	4	3	4	10	4	4	12	.	6										
15	.	2	2	8	2	1	4	7	2	1	4	7	.	.	.	.	5	5	5	5										
16	1	.	6	3	1	.	.	3	7	3	6	12	.	.	.	.	6	9	3	.										
17	4	.	12	.	4	.	.	6	4	.	.	6	.	.	12	12	.	.	.	.										
18	.	.	.	1	.	.	2	4	2	.	1	7	1	4	7	3	3	12	4	9										
19	.	.	1	.	1	.	2	7	2	.	.	4	1	2	8	2	6	14	2	8										
20	.	.	3	.	3	.	.	9	.	.	.	.	.	.	9	.	9	18	.	9										
21	.	.	6	.	6	.	6	3	6	.	.	3	3	9	.	.	.	3	3	12										
22	1	.	3	.	1	3	.	15	1	.	.	6	6	6	.	.	3	.	6	9										
23	.	.	2	2	2	.	8	1	8	.	2	7	1	6	.	1	.	4	6	10										
24	.	.	1	1	.	2	.	11	2	2	2	11	2	4	.	2	2	.	8	10										
25	.	.	2	2	2	.	8	7	8	.	2	1	3	5	2	.	4	5	1	8										
26	.	.	1	1	2	2	2	11	.	2	.	11	5	4	1	.	5	6	4	3										
27	1	.	6	3	1	3	.	12	7	.	6	3	.	.	.	.	3	.	6	9										
28	1	.	6	3	7	.	6	3	1	3	.	12	.	.	.	.	.	3	9	6										
29	.	.	6	.	6	.	6	3	6	.	.	3	.	3	3	.	3	9	3	9										
30	1	.	3	.	1	3	.	15	1	.	.	6	3	.	3	.	6	6	6	6										
31	.	.	.	6	.	.	6	3	6	.	6	3	1	4	4	1	2	8	2	8										
32	.	1	.	3	.	.	1	6	.	3	1	15	2	3	2	3	4	6	6	4										
33	.	.	2	2	2	.	8	1	8	.	2	7	.	2	5	3	1	8	4	5										
34	.	.	1	1	.	2	.	11	2	2	2	11	1	.	5	4	3	4	6	5										
35	.	.	2	2	2	.	8	7	8	.	2	1	1	.	6	1	6	10	.	4										
36	.	.	1	1	2	2	2	11	.	2	.	11	.	2	2	4	10	8	.	2										
37	.	.	6	.	6	.	6	3	6	.	.	3	.	.	9	3	3	12	.	3										
38	1	.	3	.	1	3	.	15	1	.	.	6	.	.	6	6	9	6	.	3										
39	1	.	6	3	7	.	6	3	1	3	.	12	.	3	6	9	.	.	.	.										
40	1	.	6	3	1	3	.	12	7	.	6	3	3	.	9	6	.	.	.	.										
	*	*	*		*	*	*		*	*	*	*			*			*	*		*									

TABLE 16

Parental genotype AB/Ab/Ab/aB/ab/ab, *representing a set of 2. An interchange of loci accompanied by an interchange of paired conjugate modes generates a conjugate set of 2. Row divisor : 180*

Mode of formation	Composition of gamete																			
	AB AB AB	*aB aB aB*	*ab ab ab*	*Ab Ab Ab*	*AB AB Ab*	*AB AB aB*	*ab ab aB*	*ab ab Ab*	*AB Ab Ab*	*AB aB aB*	*ab aB aB*	*ab Ab Ab*	*AB AB ab*	*AB ab ab*	*Ab Ab aB*	*aB aB Ab*	*AB Ab aB*	*ab Ab aB*	*AB aB ab*	*AB Ab ab*
1	.	.	.	.	.	.	9	18	9	.	.	18	.	9	9	.	18	36	18	36
2	.	.	12	12	12	6	12	24	12	6	12	24	12	12	12	12	.	.	.	.
3	.	.	2	2	2	.	5	16	5	.	2	16	4	15	15	4	12	34	12	34
4	.	.	12	12	12	6	12	24	12	6	12	24	.	.	.	.	12	12	12	12
5	3	3	15	15	9	3	9	21	9	3	9	21	15	15	15	15	.	.	.	.
6	.	.	2	2	2	.	5	16	5	.	2	16	6	17	17	6	10	32	10	32
7	3	3	15	15	9	3	9	21	9	3	9	21	15	15	15	15	.	.	.	.
8	.	.	.	.	.	.	9	18	9	.	.	18	6	15	15	6	12	30	12	30
9	.	.	2	2	2	.	5	16	5	.	2	16	7	18	18	7	9	31	9	31
10	3	3	15	15	9	3	9	21	9	3	9	21	.	.	.	.	15	15	15	15
11	.	.	12	12	12	6	12	24	12	6	12	24	.	.	.	.	12	12	12	12
12	.	.	.	.	.	.	9	18	9	.	.	18	6	15	15	6	12	30	12	30
13	2	2	14	14	10	4	10	22	10	4	10	22	14	14	14	14	.	.	.	.
14	.	.	2	2	2	.	5	16	5	.	2	16	2	13	13	2	14	36	14	36
15	2	2	14	14	10	4	10	22	10	4	10	22	.	.	.	.	14	14	14	14
16	3	3	15	15	9	3	9	21	9	3	9	21	.	.	.	.	15	15	15	15
17	.	.	12	12	12	6	12	24	12	6	12	24	12	12	12	12	.	.	.	.
18	.	.	2	2	2	.	5	16	5	.	2	16	5	16	16	5	11	33	11	33
19	.	.	2	2	2	.	5	16	5	.	2	16	6	17	17	6	10	32	10	32
20	.	.	.	.	.	.	9	18	9	.	.	18	9	18	18	9	9	27	9	27
21	.	.	6	6	6	.	24	12	24	.	6	12	3	9	9	3	9	21	9	21
22	.	.	3	3	3	6	3	33	3	6	3	33	6	6	6	6	15	15	15	15
23	.	.	8	8	8	.	20	10	20	.	8	10	4	12	12	4	6	22	6	22
24	1	1	4	4	2	5	2	32	2	5	2	32	8	8	8	8	14	14	14	14
25	.	.	8	8	8	.	20	10	20	.	8	10	3	11	11	3	7	23	7	23
26	1	1	4	4	2	5	2	32	2	5	2	32	7	7	7	7	15	15	15	15
27	3	3	15	15	9	3	9	21	9	3	9	21	.	.	.	.	15	15	15	15
28	3	3	15	15	9	3	9	21	9	3	9	21	.	.	.	.	15	15	15	15
29	.	.	6	6	6	.	24	12	24	.	6	12	6	12	12	6	6	18	6	18
30	.	.	3	3	3	6	3	33	3	6	3	33	9	9	9	9	12	12	12	12
31	.	.	6	6	6	.	24	12	24	.	6	12	4	10	10	4	8	20	8	20
32	.	.	3	3	3	6	3	33	3	6	3	33	7	7	7	7	14	14	14	14
33	.	.	8	8	8	.	20	10	20	.	8	10	3	11	11	3	7	23	7	23
34	1	1	4	4	2	5	2	32	2	5	2	32	7	7	7	7	15	15	15	15
35	.	.	8	8	8	.	20	10	20	.	8	10	4	12	12	4	6	22	6	22
36	1	1	4	4	2	5	2	32	2	5	2	32	8	8	8	8	14	14	14	14
37	.	.	6	6	6	.	24	12	24	.	6	12	3	9	9	3	9	21	9	21
38	.	.	3	3	3	6	3	33	3	6	3	33	6	6	6	6	15	15	15	15
39	3	3	15	15	9	3	9	21	9	3	9	21	15	15	15	15	.	.	.	.
40	3	3	15	15	9	3	9	21	9	3	9	21	15	15	15	15	.	.	.	.
	*	*			*								*	*			*			

When we take account of the gametic matrices for the five conjugate sets of genotypes (which can be developed from the matrices of tables 12-16 by an interchange of loci and of pairs of conjugate modes) there are 204 such columns. The rank of this 40×204 matrix is equal to the number of independent linear combinations of the forty frequencies which become available for estimation on classifying the gametic output of hexasomic organisms with two alleles at each of two linked loci.

Among the 147 columns marked out in the twelve gametic matrices, there are only twenty-four that are independent. Moreover, the gametic matrices for the five conjugate sets of genotypes contribute

TABLE 17

Linear combinations capable of estimation

Mode of gamete formation	Column reference numbers																							
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1	.	.	.	.	3	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
2	.	.	.	1	1	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.
3	1	.	.	.	2	.	.	.	.	.	.	.	.	.	.	.	.	2	.	.	.	.	.	.
4	.	.	.	.	.	.	.	.	.	1	1	.	1	1	.	.	.	.	.	.	.	.	.	.
5	1	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	1	3	.	.	.	.	.	.
6	2	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	2	.	.	.	.	.	.
7	.	1	1	.	1	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	3	3	.
8	.	.	.	.	1	.	.	.	2	.	.	.	.	.	.	.	.	.	.	2	.	.	.	.
9	1	.	.	.	1	.	.	.	1	.	.	.	.	.	.	.	.	2	.	1	.	.	.	.
10	1	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	1	3	.	3	.	.	.	.
11	.	.	.	.	.	.	.	1	1	.	.	.	.	1	.	.	.	.	.	4	.	.	.	.
12	3	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1
13	1	1	1	.	.	.	.	.	.	.	.	1	.	.	1	1	.	2	1	.	1	2	2	.
14	1	.	.	.	.	.	.	.	2	.	.	.	.	.	.	.	.	2	2	.	.	.	.	.
15	1	1	1	.	.	.	.	.	.	.	.	.	1	.	1	1	.	2	.	1	1	2	2	.
16	.	1	1	.	.	.	.	.	1	.	.	.	1	.	.	.	.	.	.	1	.	3	3	.
17	.	.	.	.	.	1	1	.	.	.	.	1	.	1	.	.	.	.	4	.	.	.	.	.
18	2	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	2	1	.	.	.	.	.
19	1	.	.	.	.	.	.	.	2	.	.	.	.	.	.	.	.	2	1	1	.	.	.	.
20	.	.	.	.	.	.	.	.	3	.	.	.	.	.	.	.	.	.	.	3	.	.	.	.
21	.	.	.	.	1	.	.	.	.	.	1	.	.	.	.	.	1	3	.	.	.	.	.	.
22	1	.	.	.	1	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.
23	.	.	1	.	1	.	.	.	.	.	.	.	.	.	.	.	.	4	.	.	.	1	.	.
24	2	1	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.
25	1	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	4	.	1	.	1	.	.
26	2	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	2	.	1	.	.	1	.
27	1	1	.	.	.	.	.	.	.	.	1	.	1	.	.	.	.	.	.	.	.	3	.	.
28	.	.	1	.	.	.	.	.	.	1	.	.	1	.	.	.	1	3	.	.	.	.	3	.
29	.	.	.	.	.	.	.	.	1	.	1	.	.	.	.	.	1	3	.	1	.	.	.	.
30	1	.	.	.	.	.	.	.	1	1	.	.	.	.	.	.	.	.	.	1	.	.	.	.
31	1	.	1	.	.	.	.	.	.	.	.	.	.	.	.	1	.	3	.	.	.	3	.	.
32	3	1	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	1	1	.	.	.	3	.
33	.	.	1	.	.	.	.	.	1	.	.	.	.	.	.	.	.	4	1	.	.	1	.	.
34	2	1	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	1	.	.	.	1	.
35	1	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	4	.	2	.	1	.	.
36	2	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	2	2	2	.	.	1	.
37	.	.	.	.	.	1	.	.	1	.	.	.	.	.	.	.	1	3	1	1	.	.	.	.
38	1	.	.	.	.	.	1	.	1	.	.	.	.	.	.	.	.	.	3	1	.	.	.	.
39	.	.	1	.	.	.	1	.	.	.	.	1	.	.	.	.	1	3	3	.	.	.	3	.
40	1	1	.	.	.	1	.	.	.	.	.	1	.	.	.	.	.	.	1	.	.	3	.	.

T

no further independent combination. It happens that estimates of these twenty-four linear combinations can be formed from the gametic output of genotypes belonging only to self-conjugate isomorphic sets. The following example illustrates how this can be done. The self-conjugate set of genotypes which includes bisimplex coupling $(AB)(ab)_5$, is competent to estimate the eleven linear combinations shown in the first eleven columns of table 17. The self-conjugate set of four genotypes which includes bisimplex repulsion $(Ab)(aB)(ab)_4$, also leads to estimates of eleven independent linear combinations, but of these, nine are held in common with those of the former set. The two independent combinations are shown in columns 12 and 13 of table 17. The self-conjugate set of four genotypes which includes biduplex coupling $(AB)_2(ab)_4$, contributes the seven independent combinations shown in columns 14-20, whilst the self-conjugate set of four genotypes which includes biduplex repulsion $(Ab)_2(aB)_2(ab)_2$, adds three further columns (21-23). The self-conjugate set of four genotypes which includes biduplex sub-coupling $(AB)(Ab)(aB)(ab)_3$,* yields no linear combination that is independent of the first twenty-three columns of table 17. The remaining linear combination, shown in column 24, is found in the gametic matrix for the self-conjugate pair of genotypes one of which is bitriplex coupling $(AB)_3(ab)_3$. The progression in rank of the combined gametic matrices is shown in table 18.

TABLE 18

Parental genotype	Rank of gametic matrix	Rank of combined gametic matrix
$(AB)(ab)_5$	11	11
$(Ab)(aB)(ab)_4$	11	13
$(AB)_2(ab)_4$	16	20
$(Ab)_2(aB)_2(ab)_2$	16	23
$(AB)_3(ab)_3$	10	24

The 40×16 deficiency matrix is shown in table 19. It is seen that the frequencies of only three modes of gamete formation, namely 12, 31 and 32 can be estimated singly. This deficiency matrix merits further consideration as it must contain the essentials of a system of classification of the modes of gamete formation.

5. SUMMARY

Gametic matrices are given for twelve isomorphic sets of hexasomic genotypes with two alleles at each of two linked loci. It is found that from a classification at two linked loci of the gametic output from digenic hexasomic organisms, we can estimate only twenty-four

* There are four neutral genotypes. These are duplo-triplex and comprise a conjugate pair of isomorphic sets each of two genotypes. A typical genotype is $(AB)(Ab)_2(aB)(ab)_2$.

independent linear combinations of the frequencies of the forty modes of gamete formation. The deficiency of information is therefore much greater than with two tetrasomic loci where, from doubly digenic genotypes, we can estimate ten independent linear combinations of the frequencies of the eleven modes of gamete formation.

TABLE 19

Deficiency matrix

Mode of gamete formation																		
1	.	−1	.	.	.	−1	.	.	.	.	.	.	.	.	.	.	.	
2	.	.	.	1	.	.	1	.	.	.	.	.	.	.	.	.	1	
3	.	2	.	−1	−1	3	−1	1	.	.	.	1	.	1	.	.		
4	.	.	.	.	.	.	−1	.	.	.	.	1	.	1	.	.		
5	.	.	.	−1	.	.	−1	.	.	.	.	.	.	.	.	−1		
6	−1	−2	−1	1	2	−3	1	−1	1	1	1	−1	−1	.	−1	.		
7	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.		
8	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.		
9	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.		
10	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.		
11	.	.	.	−1	.	.	.	.	.	.	.	.	.	.	.	.		
12	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.		
13	−1	.	.	.	.	.	.	.	.	.	.	1	.	1	−1	.		
14	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.		
15	1	.	.	.	.	.	.	.	.	.	.	−1	.	−1	1	.		
16	−1	.	.	.	.	.	−1	.	.	.	.	.	.	.	.	−1		
17	.	.	.	.	.	.	.	.	.	.	.	−1	.	−1	.	−1		
18	1	2	1	−1	−2	3	−1	1	−1	−1	−1	1	1	.	1	.		
19	.	−2	−1	1	.	−3	1	−1	.	.	.	−1	.	−1	.	.		
20	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.		
21	.	.	.	.	.	.	.	−1	.	.	.	.	.	−1	.	.		
22	.	.	.	.	.	.	.	.	−1	.	.	−1	1	−1	1	−1		
23	.	.	.	.	.	.	.	.	.	−1	.	.	.	.	.	.		
24	.	.	.	.	.	.	.	.	.	.	−1	.	.	.	.	.		
25	.	.	.	.	.	.	.	.	.	.	.	−1	.	.	.	.		
26	.	.	.	.	.	.	.	.	.	.	.	.	−1	.	.	.		
27	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.		
28	.	.	.	.	.	.	1	.	.	.	.	.	.	.	−1	1		
29	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.		
30	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.		
31	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.		
32	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.		
33	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.		
34	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.		
35	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.		
36	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.		
37	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.		
38	.	.	.	.	.	.	.	.	.	.	.	1	−1	1	−1	1		
39	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.		
40	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1		

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DOUBLE REDUCTION AT THE *MID* LOCUS IN *LYTHRUM SALICARIA*

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I. INTRODUCTION

THE gametic output from polysomic organisms of various genotypes can be expressed in terms of a single parameter, namely, the frequency of double reduction. This is the frequency with which a gamete receives two alleles derived from the same chromosome at meiosis.

The term " double reduction " was first introduced by Darlington (1929) and defined as a case " where both chromatids of one chromosome pass into the same nucleus at the second telophase ". This offered an explanation for the appearance of one recessive in a progeny of 137 from a reciprocal cross between a triplex and nulliplex Dahlia obtained by Lawrence (1929). Similar results had been obtained by Blakeslee, Belling and Farnham (1923), who got 2 per cent. recessives from a triplex Datura when backcrossed to the recessive, and 1 per cent. when crossed to the simplex genotype. These early workers, however, expected to find an abnormal number of chromosomes in the recessives. Blakeslee *et al.* examined their recessives but found the numbers to be normal.

Haldane (1930) pointed out that though the passing of two identical chromatids to the same gamete in a diploid organism implied an abnormal chromosome complement in the resulting zygote, this was not the case in polyploids. He calculated the ratios to be expected if the eight chromatids of a tetraploid were distributed at random into the gametes. In the mating between a triplex and a nulliplex, one recessive in 28 could be expected on this hypothesis. Darlington (1931), however, showed that cytologically there were a number of restrictions to Haldane's theory, and concluded that intermediate conditions between random segregation of chromatids and diploid segregation were to be expected, and had in fact been observed by Lawrence.

In four crosses involving one triplex with four different nulliplex, Lawrence (1935) observed double reduction at the Y locus in tetraploid Dahlia. He obtained 7 recessives out of 385 plants and explained this as " probably due to chromatid segregation ". At none of the three other loci studied did double reduction appear. It should be noted, however, that it was never certain that a triplex was involved in the matings.

An important contribution was made by Mather (1936) when he

pointed out that " the amount of double reduction is a function of the cross-over distance between the locus and the spindle fibre," and, therefore, will vary from locus to locus. This has since been demonstrated in *Lythrum salicaria* by Fisher (1949), who gave preliminary estimates of the frequency of double reduction at the purple/pink locus as 6·5 and at the short/non-short locus as 2·5. These estimates were obtained incidentally in an attempt to ascertain the frequencies of the eleven modes of gamete formation now known to be characteristic of linkage in tetrasomic organisms. They are based on the proportion of duplex plants obtained from simplex parents backcrossed to the recessive long-styled pink. This method involves making a second backcross to determine the genotype—whether duplex or simplex—of the dominant offspring.

An estimate based on the proportion of recessives obtained on backcrossing the triplex genotype is more direct, requiring no second backcross and can, in consequence, be based on large numbers with greater facility. This latter method has been used to estimate the frequencies of double reduction both in pollen and ovules at the mid locus in the work described here. It should be noted that for an estimate at the short locus this method cannot at present be used, since a triplex short could only be obtained by illegitimate fertilisation. All attempts to obtain such a plant have so far failed.

2. DOUBLE REDUCTION IN OVULE FORMATION

When Fisher and Mather (1943) established the existence of polysomic inheritance in *L. salicaria*, they did so by backcrossing to the long-styled form, 16 Shorts and 12 Mids obtained from a cross between a Short and a Mid, both of which had one and the same dominant mid allele. The resulting progenies showed that one Short was nulliplex for mid, 11 were simplex and 4 duplex ; 8 of the Mids were simplex, 3 duplex and 1 plant, M40·2F, gave no Longs out of 41 non-shorts. This plant was tested the next year with a progeny of 1034 (Fisher, 1944). It gave 22 Longs out of a total of 1075 in two years, and must, therefore, be a triplex and have arisen by double reduction. This first evaluation of the frequency gave an estimate of 8·19 $\pm$1·73 per cent.

In an attempt to obtain a Short triplex for mid, two of the Shorts that were found to be duplex for mid were crossed to a simplex Mid ($Ss_3\,M_2m_2 = s_4\,Mm_3$), and twenty-five Shorts obtained from this cross were tested in 1945. The twenty-five progenies are given in table 1, from which it will be seen that five plants triplex for mid were identified. Fifteen of these plants were again backcrossed, this time to pale pinks to provide triplex pales and data on the linkage of the Short and purple loci. The test progenies grown in 1946 are given in table 2, in which the Long, Mid and Short segregations alone are given. There is evidence that some stray pollen was present from the

2 Mids that appeared in the progeny of the nulliplex plant. The data from the four triplex plants will nevertheless be included in our estimate of double reduction, for, as will be shown later, a small percentage of foreign pollen does not alter the estimate appreciably. The fifth triplex plant was crossed to the triplex Mid, M40·2F, in an attempt to obtain both Shorts and Mids quadruplex for mid. In 1947 eleven

TABLE 1

Test progenies from 25 Shorts (1945)

Genotype	Plant	L	M	S	Total
Nulliplex	S44·2G15	105	...	89	*194*
Simplex	S44·2F1	30	63	105	*198*
	S44·2F2	39	62	97	*198*
	S44·2F3	47	47	107	*201*
	S44·2F4	38	60	101	*199*
	S44·2F5	52	55	95	*202*
	S44·2F6	39	43	114	*196*
	S44·2G1	41	57	102	*200*
	S44·2G5	55	62	84	*201*
	S44·2G8	30	51	123	*204*
	S44·2G16	57	51	88	*196*
	S44·2G18	36	42	84	*162*
	Total	**464**	**593**	**1100**	**2157**
Duplex	S44·2F7	12	94	95	*201*
	S44·2G3	14	90	96	*200*
	S44·2G4	8	91	102	*201*
	S44·2G6	13	85	105	*203*
	S44·2G7	16	68	114	*198*
	S44·2G9	21	71	109	*201*
	S44·2G10	18	85	98	*201*
	S44·2G14	10	83	96	*189*
	Total	**112**	**667**	**815**	**1594**
Triplex	S44·2G2	2	95	105	*202*
	S44·2G11	1	87	111	*199*
	S44·2G12	4	89	106	*199*
	S44·2G13	4	93	100	*197*
	S44·2G17	1	88	109	*198*
	Total	**12**	**452**	**531**	**995**

Shorts and twelve Mids obtained from this cross were backcrossed to Longs and progenies grown in 1948. It will be seen from the square-root chart in fig. 1 that five Shorts and six Mids triplex for mid were found.

The possibility that the eleven plants classified as triplex were not all that should have been so classified, *i.e.* that one or more had been classified as quadruplex, may be examined as follows. The number of Long progeny observed from nearly equal progenies with the same

frequency of double reduction, should be distributed nearly in a Poisson Series. If m is the expectation, then the frequency of cases in which none occur will be e^{-m}, and the frequency of cases with at least one

TABLE 2

Test progenies from 15 Shorts (1946)

Genotype	Plant	L	M	S	Total
Nulliplex .	S44·2G15	48	2	63	*113*
Simplex .	S44·2F3	42	44	101	*187*
	S44·2G1	32	41	93	*166*
	S44·2G5	35	51	95	*181*
	S44·2G8	29	55	92	*176*
	S44·2G16	34	40	108	*182*
	S44·2G18	42	44	101	*187*
	Total . .	**214**	**275**	**590**	**1079**
Duplex .	S44·2G7	9	68	95	*172*
	S44·2G9	13	71	108	*192*
	S44·2G10	9	72	108	*189*
	S44·2G14	6	60	69	*135*
	Total . .	**37**	**271**	**380**	**688**
Triplex .	S44·2G11	3	47	46	*96*
	S44·2G12	2	64	110	*176*
	S44·2G13	3	80	98	*181*
	S44·2G17	...	79	70	*149*
	Total . .	**8**	**270**	**324**	**502**

will be $1-e^{-m}$. Among these latter the average number of Long plants observed must be $m/(1-e^{-m})$. Actually the average number observed among the 11 progenies classified as from triplex seed parents

TABLE 3

Double reduction in ovule formation

Year grown	Progenies	L	M	Total
1942 and 1943 .	M40·2F	22	1053	*1075*
1945 . . .	5 Shorts	12	452	*464*
1946 . . .	4 Shorts	8	270	*278*
1948 . . .	5 Shorts	16	418	*434*
	6 Mids	15	458	*473*
1946-1951 . .	S45·2	5	267	*272*
	Total . .	**78**	**2918**	**2996**

is 31/11 or 2·8̇1̇. This corresponds with an estimated m of 2·61114 for which e^{-m} is 0·07345. The number of triplex progenies corresponding with 11 observed is, therefore, 11/0·92655, or 11·872. If, therefore,

we add 7/8 of the average number of plants in a progeny, *e.g.* about 70, to the number of plants not Long observed in the progenies, *i.e.* if we take an estimate based on 31 out of 977 instead of 31 out of 907, we shall have made a reasonable allowance for the possibility that a triplex progeny has been excluded. The correction arrived at is so trifling, that more elaborate calculations are not necessary.

Another triplex, S45·2, was identified from a repetition in 1944 of the cross, $Ss_3\ M_2m_2 = s_4\ Mm_3$, made the previous year. Progenies

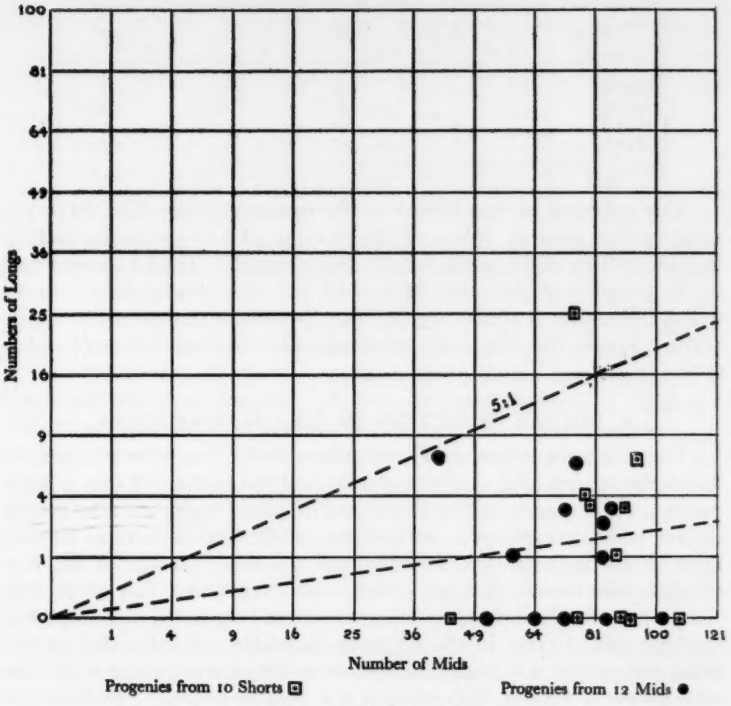

FIG. 1.—1948 test progenies from $Ss_3\ M_3m = s_4\ M_3m$

from this plant have been grown repeatedly since then. The results are shown in the fifth line of table 3, in which all the data bearing on ovule formation are summarised. The percentage of Longs in the progenies—2·6035 per cent. over all—represents a quarter of α, the frequency of double reduction, in ovule formation, which is, therefore, estimated at 10·414 per cent. If we include the allowance made on the 1948 progenies, the percentage of Longs is 2·544, giving an estimate for $\alpha = 10{\cdot}176$ per cent. Both the crude and the corrected data are homogeneous. In the first case $\chi^2 = 4{\cdot}393$ and in the second $\chi^2 = 3{\cdot}304$ for 4 degrees of freedom.

3. SHORTS AND MIDS QUADRUPLEX FOR MID

Amongst the plants tested in 1948, four Shorts and five Mids gave no Longs in their progenies (fig. 1). Four of these plants, two Shorts and two Mids, were tested again in 1949 with larger progenies. The combined data from both years are given in table 4.

TABLE 4

Test progenies from 2 Shorts and 2 Mids quadruplex for Mid

Plant	L	M	S	Total
S47·16 . .	...	552	575	*1127*
S47·23 . .	...	571	582	*1153*
M47·8 . .	1	546	...	*547*
M47·12 . .	...	567	2	*569*

The presence of two Shorts in the progeny of the Mid, M47·12, must be due to stray pollen at the crossing plot or to contamination in the handling of the seeds, which are very small. The Long observed in the progeny of the other Mid must also be a contaminant, since, if this Mid were a triplex 15·65 Longs would be expected from 547 plants. It was, therefore, concluded that all four plants are quadruplex for mid.

4. DOUBLE REDUCTION IN POLLEN FORMATION

To obtain an estimate of α in pollen formation it was necessary to use the triplex Mid as pollen parent and the recessive Long as seed parent. As demonstrated by Fisher and Mather (1943), the advantages of the technique of open pollination, which we have used for all tests in this investigation, far outweigh the disadvantage of the risk of contamination by foreign pollen, and they found this to be less than 1 per cent. in their progenies. It should be pointed out, however, that the risk of error in the estimate of double reduction due to the same percentage of foreign pollen is about fifty times greater when the seed parent is a Long than when it is a Mid or Short. This becomes apparent when it is realised that all the ovules from a Long will, if fertilised by pollen carrying neither mid nor short, increase the proportion of long-styled individuals in the progeny. Since calculation shows that 42 per cent. of such pollen exists in a theoretical population (Fisher, 1941), even 5 per cent. stray pollen at the crossing plot would almost double the percentage of Longs observed in the progeny. On the other hand, when the triplex Mid is the seed parent, only those ovules not carrying mid, that is about 2 per cent., can if fertilised by pollen carrying mid, result in a deficiency of Longs in the progeny. If 5 per cent. foreign pollen were present, the deficiency would be less than 0·05 per cent., a difference that could make no serious difference to the estimate of α.

With this very real source of error in mind, great care was taken in 1948 to find a plot far from any known Lythrum populations for the pollination of a long-styled plant by the triplex Mid, M40·2F. To make plenty of the right pollen available, the Mid was divided and planted in a ring round the Long. Seed taken from the Long was grown first in 1949 and again in 1951. The data are given in table 5.

TABLE 5

Double reduction in pollen formation

Year	L	M	S	Total
1949 . .	10	374	...	*384*
1951 . .	21	943	...	*964*
Total . .	**31**	**1317**	...	**1348**

The percentage of Longs—2·300 per cent.—is slightly less than that observed in ovule formation. This, and the fact that no Shorts were observed in either year, is convincing evidence that foreign pollen was absent at the crossing plot. The estimate of a is 9·199 per cent. The data for both years are homogeneous—$\chi^2_{(1)} = 0{\cdot}2225$.

The data from all years, 1943-1951, from both ovules and pollen combined are homogeneous, $\chi^2 = 5{\cdot}110$ for 6 degrees of freedom, indicating that seasonal variations do not affect the frequency of double reduction. Taking the two estimates together we get $2{\cdot}509 \pm {\cdot}237$ per cent. Longs and $a = 10{\cdot}037 \pm {\cdot}949$ per cent., and $\chi^2 = 0{\cdot}351$ for 1 degree of freedom, on the crude data. If we include a twelfth progeny from the 1948 families, the percentage of Longs is $2{\cdot}469 \pm {\cdot}234$ per cent., giving $a = 9{\cdot}878 \pm {\cdot}936$ per cent., and $\chi^2 = 0{\cdot}232$ for 1 degree of freedom. Our estimate is only slightly higher than that forecast by Fisher and Mather in 1943.

I am much indebted to Professor Sir Ronald Fisher, not only for his constant guidance and help in every aspect of the work, but for supplying the material and suggesting the investigation.

5. SUMMARY

About the time when it was realised that the gametic output from polysomic organisms of various genotypes could be expressed in terms of a single parameter, viz. the frequency of double reduction, a, an attempt was first made to estimate this frequency at the mid locus in *Lythrum salicaria*. When Fisher and Mather (1943) established polysomic inheritance in *L. salicaria*, they obtained a triplex mid that could only have arisen by double reduction. This plant was backcrossed to the recessive long-styled form and in two seasons gave 22 Longs out of 1075. The proportion of Longs represents a quarter of a in ovule formation, which was then estimated at 9·18 per cent. Similar

data has been obtained in subsequent years. In 1948 triplex and quadruplex plants were distinguished only by their performance in producing or not producing Longs. Allowance has been made for the possibility that one or more triplex had been classified as a quadruplex by adding 7/8 of the average number of plants in a progeny, 70, to the Mids observed. The corrected total of 78 out of 3066 plants were obtained in five years. The proportion of Longs is 2·544 per cent. giving $\alpha = 10{\cdot}176$ per cent.

To obtain an estimate of α in pollen formation the reciprocal cross was made in 1948. In such a cross the risk of error from contamination by foreign pollen is about 50 times greater than when the triplex plant is the seed parent. Since the open pollination technique was used throughout, special care was taken in selecting a site for the pollination plot for this cross. In two years 31 Longs were obtained out of 1348 plants ; no Shorts were observed, thus providing evidence that foreign pollen was absent. The percentage of Longs is 2·300 per cent., giving an estimate of $\alpha = 9{\cdot}199$ per cent. in pollen formation, very close to the value for ovules. Combining the data from ovules and pollen the proportion of Longs is $2{\cdot}469 \pm {\cdot}234$ per cent., and $\alpha = 9{\cdot}878 \pm {\cdot}936$ per cent. The data obtained from 1942 to 1951 are homogeneous, $\chi^2_{(6)} = 5{\cdot}110$, indicating that α is not affected by seasonal differences.

Of the Shorts and Mids likely to be quadruplex for mid, two of each type have been tested with adequate numbers and their genotype verified. All four were morphologically normal showing the completeness of dominance at the mid locus.

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REVIEWS

QUANTITATIVE INHERITANCE. Papers read at a colloquium held at the Institute of Animal Genetics, Edinburgh University, under the auspices of the Agricultural Research Council, 4th to 6th April 1950. Her Majesty's Stationery Office, London, 1952. Price 20s. net.

It was enterprising of the Agricultural Research Council to organise and to publish the contributions to this symposium on *Quantitative Inheritance*. Nearly all the contributors stress the importance of quantitative characters in evolution and in animal and plant improvement, and this represents a real advance from the attitude of the first generation of geneticists, about 1910, though it is no novelty for the present generation.

Like most such occasions, this discussion suffered to some extent from the reiteration by the older hands of opinions of which they have ceased to be critical, and by the naive misapprehensions of the newcomers. Nevertheless, some material of interest has been gathered.

Attention may be drawn to the short paper by L. L. Cavalli, "An analysis of linkage in quantitative inheritance" using the methods and material of K. Mather. Instructive also is Mather's gentle but effective reproof of the extravagances of a purely negative contribution by B. Woolf.

The paper, however, from which most can be learnt, and which is the chief justification for the publication, is that of Mather and Vines on "The inheritance of height and flowering time in a cross of *Nicotiana rustica*." This sets out fully and usefully the analysis up to the fourth generation, with a valuable discussion of those factors, such as scaling, which help to make the analysis more accurate. R. A. F.

HEREDITY IN UTERINE CANCER. By Douglas P. Murphy. Harvard University Press (London : Geoffrey Cumberlege). 1952. Pp. xi+128. 16s.

Although geneticists on the whole incline to the view that a predisposition to cancer is inherited, the opinions held by many authorities who have studied the subject are somewhat diverse. With the possible exception of the work of Brøbeck, the existing literature seems to lack any really satisfactorily comprehensive data on the familial occurrence of cancer, involving in particular an adequate series of controls. The present book describes the methods and results of an enquiry whose purpose was to make good this deficiency. It is concerned with the occurrence of cancer among the female relatives of 201 women suffering from cancer of the uterine cervix and of 215 controls. In the first section of the book the literature is reviewed and discussed, and emphasis is laid on the need for a new survey. The second section deals with the materials and methods employed, and is a model of clarity and precision. Full details are given of the purpose of the investigation, the use made of a pilot study, the selection of both the cancer and control probands, the methods of obtaining and handling the clinical and social information required, personnel employed, and so on. The results achieved are elaborated in the third section and discussed in the fourth. Certain details of the administrative background and the questionnaires used are presented in appendices.

The general conclusions of the survey are that hereditary factors do influence the frequency of occurrence of uterine cancer, though the effect is small. Thus the ratio of cases of uterine cancer to all cases of cancer was 26 per cent. in the female relatives of cancer probands, compared

with about 15 per cent. in the relatives of the control probands. Again, 3·2 per cent. of the mothers and aunts of cancer probands were recorded as having cancer of the uterus, compared with 1·4 per cent. of the mothers and aunts of the controls. The total prevalence of cancer at some site did not differ between the cancer and control families. Finally, excluding the proband-mother relationships, it is shown that 9 per cent. of the mothers of non-cancerous daughters had cancer in some site, compared with a figure of 17 per cent. for mothers of daughters with cancer irrespective of site ; whereas only 9 per cent. of the mothers of the cervical cancer probands had cancer in some site. This seems to imply that genetic factors other than those concerned with uterine cancer affect the occurrence of cancer elsewhere.

The chief criticism which must be made of the treatment is that most of the tables presented are simple one-way analyses of material relating either to the cancer family groups or to the control family groups. No attempt has been made to examine two- or three-way tables. This is important as there are, for example, marked differences in the economic and educational levels of the cancer probands compared with the controls (and perhaps their families as well). Thus some of the apparent differences in the prevalence of cancer referred to above might be ascribable to purely environmental factors. However, in spite of this qualification, it is true to say that Dr Murphy's book does make an extremely useful contribution to the study of the ætiology of cancer, and is well worth the serious attention of clinicians and geneticists alike. NORMAN T. J. BAILEY.

STATISTICAL METHOD IN BIOLOGICAL ASSAY. By D. J. Finney. Griffen. 1952. xix+661 pp. 68s.

Frequently it happens, whether from necessity or convenience, that we wish to measure the potency of some substance or stimulus, which may be chemical, physical, psychological, etc., by means of the effects produced in living subjects. This is the field of biological assay, which, at least so far as quantitative methods are concerned, is extremely susceptible of considerable statistical development. The great advances of recent years in both the scope of bioassay itself and in the statistical design and analysis of the experimental procedures have been evolved mainly in relation to pharmacology. Thus one of the most convenient accounts of the statistical methods available was, until the publication of the present book, the third chapter (occupying more than a third of the whole volume) by Dr Finney in the second edition of *Biological Standardization* by Burn, Finney and Goodwin. However, there are immediate applications to other subjects like psychology or physiology, where, using similar experimental methods, interest is centred in the living subjects, rather than in the stimuli themselves. Again, methods of analysis may be used elsewhere because of similarities in the underlying mathematical model. There is for example the application of the modified form of probit analysis, used when there is an independent source of natural mortality, to the problem of estimating the average age of onset of a chronic disease from its prevalence in different age groups.

In the present book Dr Finney has, in his own words, " attempted to provide a comprehensive account of designs and statistical analyses for biological assays, both as a textbook for the student of statistics and as a work of reference for the practitioner of bioassay." This aim has been

largely fulfilled. Assuming a certain acquaintance with basic statistical methods, a wide variety of problems peculiar to assay work is introduced, and the appropriate analytical procedures are explained, illustrated and discussed. Thus there are chapters dealing with parallel line assays; slope ratio assays; quantal responses; symmetrical designs; efficiency, reliability and sensitivity; incomplete block designs; cross-over designs; use of concomitant information; combination of estimates, etc., etc. There is also a fairly extensive bibliography and seventeen appendix tables giving the distributions of t, χ^2, Fisher-Behrens ratio and variance ratio, as well as all the usual tables of transformations, working values, ranges, and weighting coefficients, for probits, logits, angles and loglogs. A great many general statistical techniques are introduced into the text and full explanations are given of their application and interpretation. As a result, the book is a more or less self-contained handbook on the subject, valuable as a work of reference both to the practitioner of bioassay and to the student of the primarily statistical aspects. One useful feature is the inclusion of four suggested alternative selections of sections from the book designed to meet the needs of the professional statistician desiring a short course in biological assay, the non-mathematical user of assay, the reader requiring a general survey of the function of statistics in the subject, and the reader who is mainly interested in work with quantal responses.

The general excellence of *Statistical Method in Biological Assay* justifies, in the opinion of the reviewer, the somewhat high price. Because of its comprehensive treatment and lucid exposition, Dr Finney's book deserves to be widely read and to become a standard work of reference in the field of bioassay.

NORMAN T. J. BAILEY.

EVOLUTION IN THE GENUS *DROSOPHILA*. By J. T. Patterson and W. S. Stone. New York: The Macmillan Company. 1952. Pp. 1-599. $8.50.

The evidence of evolutionary processes is at once more comprehensive and more significant in *Drosophila* than in any other group of animals or plants. It is derived from comparative anatomy, geographical distribution and ecology, combined with breeding and chromosome studies of many hundreds of species and of races in all parts of the world. This book is an account of the evidence accumulated, chiefly since 1910, to which the authors and their collaborators have so largely contributed.

Each of the different aspects of *Drosophila* evolution is considered in some detail and particularly later in relation to the bases of genetic isolation. In general the authors keep their eyes fixed on one problem at a time and they do not allow themselves to be distracted, by what seem irrelevant clues, from their appointed programme. This is a successful policy up to a point. But, beyond that point, the desire to solve problems which were envisaged at the beginning suppresses the tendency for new problems to push their way into the discussion.

Moreover, we find that, in keeping with this attitude, the text has been put together in detail as though from an index of notes or reprints classified chiefly under the names of authors. Nearly half the paragraphs begin with these names (sometimes three or four of them): the connexion between such paragraphs will not be clear to the reader unacquainted with the hundreds of interesting personalities mentioned. On a larger scale the order of the book is confused in another way. For example there are 56 pages of sporadic references to salivary gland chromosomes before

the authors take the plunge and tell us how the meaning of these chromosomes was discovered. Even so they do not tell us the part played in this by Heitz and Bauer who arrive on the scene a year late.

Such discontinuities of style and arrangement are not unrelated to the theoretical character of the book. " Natural Selection " appears (p. 264) as a sub-heading between, and on an equal footing with, " Gene Homologies " and " Genic Balance ". " Genetic system " is equated with " genome " (p. 112) : whether the same equation holds later (on p. 537) is doubtful. But perhaps most serious of all is the authors' inevitable failure to take advantage of the specific opportunities of discovering fundamental principles from these very experiments with *Drosophila*.

Of the 121 diagrams which illustrate types of chromosome complement in the male, about 30 per cent. reveal no difference between X and Y chromosomes. In some instances as an afterthought the authors call attention to this apparent identity, *e.g.* " The largest V is the X chromosome ; and the Y is also a V, which is indistinguishable from the X " (p. 147). Now evolution in the sex chromosomes is clearly rapid in *Drosophila* : four species are represented as having no Y at all in the male ; others have two X's. Certain questions therefore arise. Are the two apparently identical sex chromosomes a newly arisen system with a single gene difference ? Or are there large but equal-sized differential segments in X and Y ? Or has the female become the heterozygous sex in these races or species ?

To all these questions the salivary gland chromosomes and the distribution of heterochromatin might provide answers. But these matters are not brought into relation. Indeed, in the enormous index, sex chromosomes, or chromosomes : sex, or crossing-over, or linkage, or differential segments, do not appear.

This book, it is to be feared, will not be attractive to the general student : at least not so attractive as the importance of its theme suggests or deserves. Nevertheless it should fulfil a special purpose. For one who knows what he wants to find out from *Drosophila*, one who is already familiar with its genetical history and uses, and with the problems of evolutionary genetics in general, for such a one the references, tables, figures, diagrams and maps will suggest any number of new enquiries. C. D. D.

GENETICS FOR SCHOOLS. By Prof. K. Mather, F.R.S. Modern Science Memoirs, No. 31. London : Published for the Science Masters' Association by John Murray. Pp. 36. 1s. 6d.

A valuable first year's course in all aspects of genetics : plant and animal, cytological and physiological, practical and theoretical.

THE BIOLOGY OF MENTAL HEALTH AND DISEASE. With 108 Contributors. Foreword by Stanley Cobb, M.D. London : Cassell. Pp. i-xvii+654. 75s.

This book contains 38 articles, two of which, by F. J. Kallmann and R. R. Gates, and a discussion of mongolism, are of genetic interest.

NEW JOURNALS

JOURNAL DE GÉNÉTIQUE HUMAINE. No. 1 of Vol. 1 published June 1952. Genève : Médecine et Hygiène. (4 numbers per annum : Fr. fr. 3000 p.a.)

ACTA GENETICAE MEDICAE ET GEMELLOLOGIAE. No. 1 of Vol. 1 published January 1952. Rome : Largo Dell'Amba Aradam, 1. £5 p.a.

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